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Vol. 37, No. 5 Transactions, American Geophysical Union October 1956

# Variations in the Equatorial Countercurrent in the Central Pacific

T. S. AUSTIN, E. D. STROUP, AND M. O. RINKEL

*Abstract*—Cruise 15 of the M.V. *Hugh M. Smith* consisted of four consecutive lines of oceanographic stations along 140°W longitude between 8°N and 7°S latitude. Geostrophic calculations show that during a five-week period the maximum eastward velocity component in the Countercurrent progressively increased from 63 to 120 cm/sec and the volume of flow increased from 18.3 to 22.6 × $10^6$ $m^3$/sec. In spite of these short-term variations, the Equatorial Countercurrent in the central Pacific is remarkably predictable in latitude and general physical characteristics.

The Pacific Oceanic Fishery Investigations (POFI) of the U. S. Fish and Wildlife Service has been taking oceanographic observations in the central equatorial Pacific since early 1950 [*Sette*, 1954]. This program has been designed to describe the marine physical, chemical, and biological environment, particularly as it relates to the abundance and distribution of tuna. Early oceanographic surveys were planned to provide an understanding of the general circulation features; subsequent studies have been designed to investigate the regions and features thought to be of particular biological importance. *Stroup* and *Austin* [1955] have reviewed the POFI oceanographic programs and list the publications resulting therefrom.

The almost 100 meridional crossings of the central Pacific equatorial currents by POFI vessels demonstrate the over-all persistence of the zonal circulation and show evidence of superimposed transients. Cruise 15 of the POFI oceanographic research vessel, *Hugh M. Smith*, was specifically designed to investigate these short-term variations along 140°W longitude by completing four consecutive station lines; the observed data from this cruise along with the meridional sections and a brief descriptive text were published by *Austin* [1954]. Our paper is based primarily on these data.

For orientation during the following discussion, a typical meridional temperature section across the major equatorial currents is shown in Figure 1. A temperature section from Cruise 11 was chosen for Figure 1 as the similar sections from Cruise 15 did not penetrate the North Equatorial Current. The well-developed 'two-layered' system characteristic of these latitudes throughout the year is evident. The configuration of the isotherms reflects the mass distribution associated with the zonal flow. The Equatorial Countercurrent, as shown by the southward deepening thermocline, flows east (out of the section) between 10°N and 5°N latitude; the west-setting North and South Equatorial Currents lie to the north and south of the Countercurrent with the South Equatorial Current on both sides of the equator. The lower temperatures of the surface layer about the equator are indicative of the divergence and associated enrichment from below, a biologically significant feature of the central equatorial Pacific [*King* and *Demond*, 1953; *Murphy* and *Shomura*, 1953].

It has been shown by *Montgomery* and *Palmén* [1940] that the most probable explanation for the Countercurrent is the downward west-east slope of the sea surface resulting from the westerly stress of the trade wind system. They demonstrate that the Countercurrent is a 'downhill' return flow in the region of the doldrums; here the NE and SE trades converge and rise, resulting, climatologically, in light and variable winds. In the central Pacific between approximately 110°W and 170°W longitude, the mean positions of the doldrums and the Countercurrent agree very closely and exhibit similar seasonal meridional fluctuations, whereas east and west of this region the situation appears more complex and the relationship between wind stress and Countercurrent requires further investigation.

At the time of Cruise 15 (June, 1952) the mean positions of the subtropical anticyclones were as shown in the left-hand panel of Figure 2. The station lines (heavy vertical line) approximately straddle the mean position of the equatorial pressure trough during this period. Normally, during the month of June, the trough is somewhat farther north, centered at 7° to 9°N latitude [*Riehl*, 1954].

The wind direction and speed observed on each of the four legs during Cruise 15 are shown in the right-hand panel of Figure 2. The direction scale centers on 090°T; thus the portion of the curve to the left of center represents winds from the northeastern quadrant, that to the right, winds

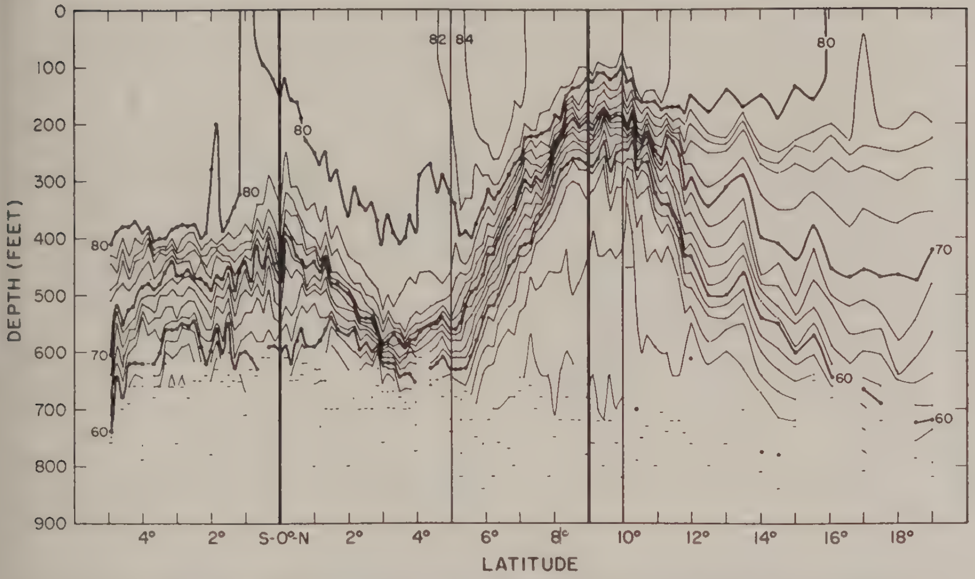
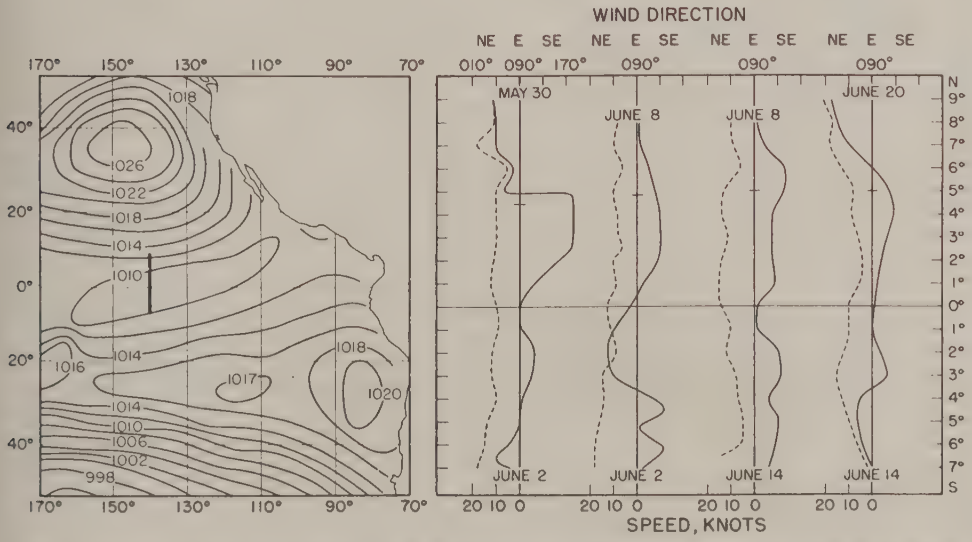


Fig. 1 – Vertical meridional distribution of temperature (°F) from *Hugh M. Smith* Cruise 11, 150°W longitude, September 25 to October 4, 1951; the bold vertical lines denote the meridional limits of the *Hugh M. Smith* Cruise 15 profiles in this paper; the light vertical lines show the computed boundaries of the Equatorial Countercurrent for Cruise 11


170°
150°
130°
110°
90°
70°
40°
20°
0°
20°
40°
1018
1026
1022
1018
1014
1010
1016
1014
1017
1018
1020
1014
1010
1006
1002
998
WIND DIRECTION
NE E SE NE E SE NE E SE NE E SE
010° 090° 170° 090° 090° 090°
MAY 30
JUNE 8
JUNE 8
JUNE 20
JUNE 2
JUNE 2
JUNE 14
JUNE 14
N
9°
8°
7°
6°
5°
4°
3°
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5°
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S
20 10 0
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20 10 0
SPEED, KNOTS

Fig. 2 – (Left panel) Mean atmospheric pressures in mb for June 1952, the positions of the Cruise 15 sections are shown by the heavy black line; (right panel) the true wind direction (solid line) and wind speed (broken line) observed on Cruise 15; both curves are a sighted mean drawn through observations taken at ten-mile intervals; the observed position of the southern boundary of the Countercurrent is shown for each leg by the horizontal dash; the northern boundary was at approximately 10°N, the exact position cannot be determined from the data; the data for the mean June pressure distribution in the northern hemisphere are taken from *U. S. Weather Bureau* [1952]; data for the southern hemisphere are from microfilm records provided by C. E. Palmer, Oahu Research Center, Institute of Geophysics, University of California

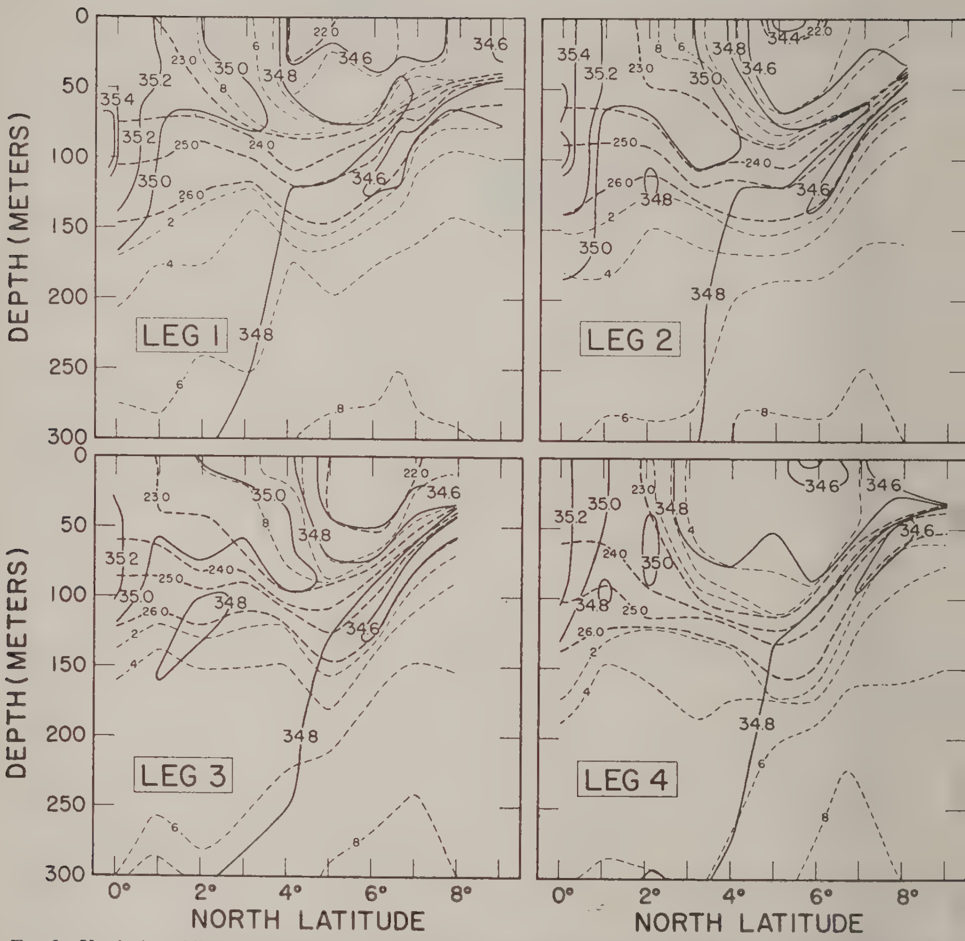

Fig. 3 – Vertical meridional distribution of salinity in per mille (solid) and sigma-t (dashed) on each leg of Cruise 15

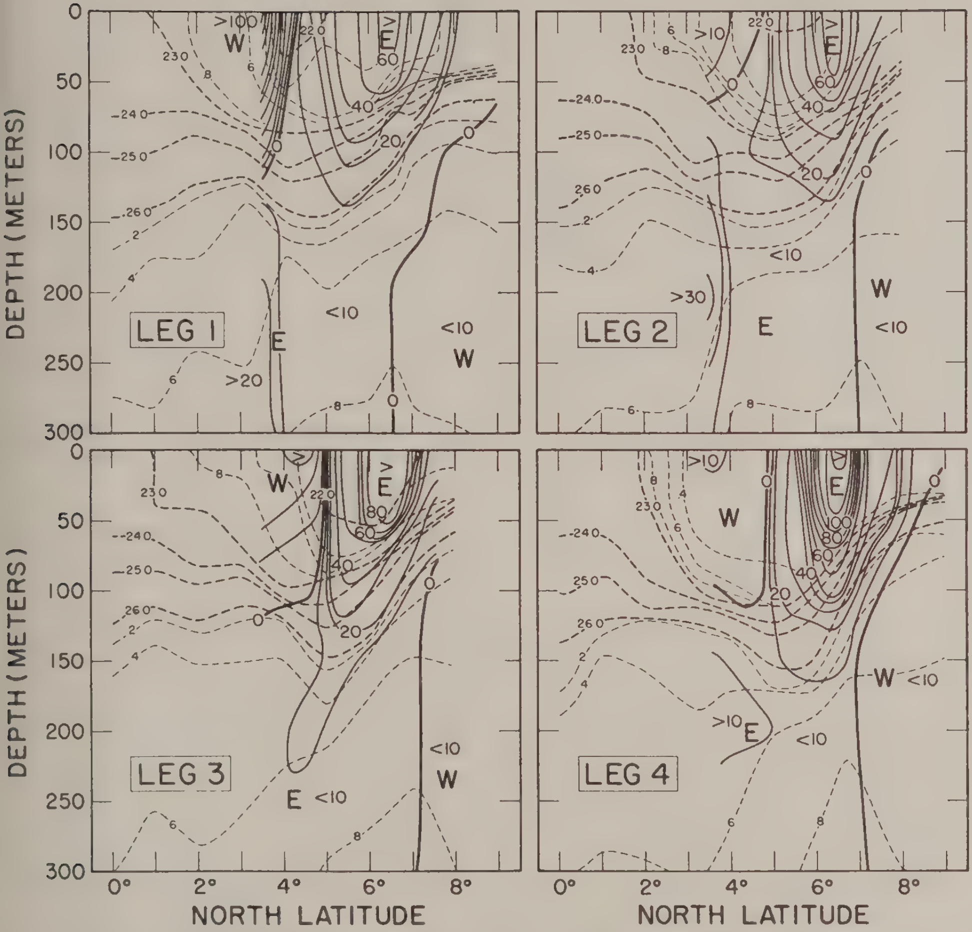

FIG. 4 – Vertical meridional distribution of currents in cm/sec relative to the 400-db level (solid) and sigma-t (dashed) on each leg of Cruise 15

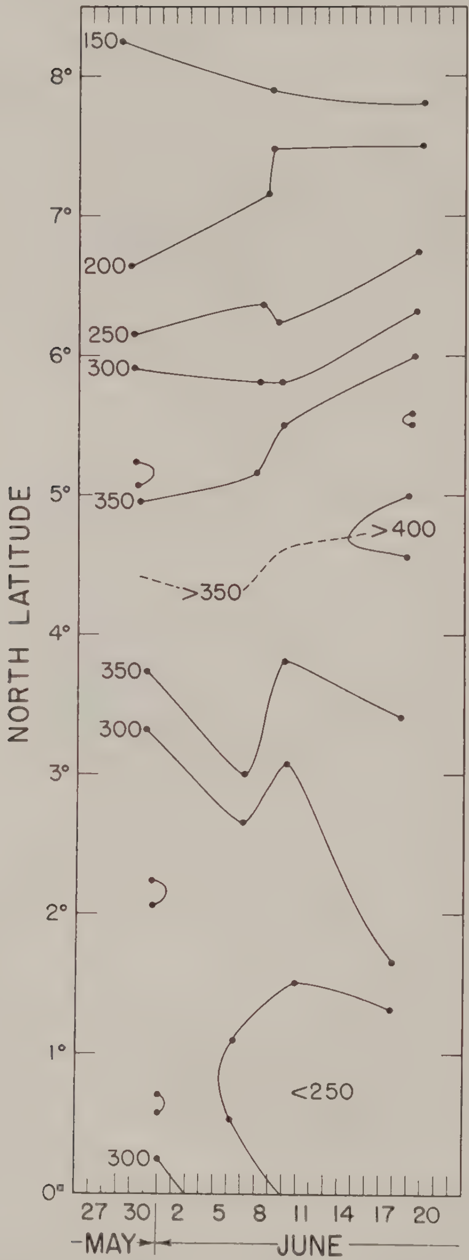

Fig. 5 – Time-latitude plot of depth of the 70°F isotherm; dashed line indicates center of trough at southern boundary of Countercurrent

from the southeastern quadrant. The classical picture of the doldrums is lacking, although there is convergence between the northeast and southeast trades at about 5° or 6°N on the first and fourth legs. At the northern end of the second and third legs the wind direction tends to be easterly.

Variations in the oceanographic features north of the equator are illustrated in Figures 3, 4 and 5. The distribution of salinity superimposed on the sigma-t field (Fig. 3) shows the normal features of the salinity field for this region; these include the northern limit of the salinity maximum of the South Pacific, the region of low salinity at the surface in the Countercurrent, and the southernmost extent of the North Pacific salinity minimum, which here is relatively shallow, lying in the lower part of the thermocline. The South Pacific maximum lying in the center of the thermocline ends abruptly near the equator, though some of the more salty water from the south continues northward in the lower part of the surface layer and upper thermocline.

The changes in salinity are largely due to advection and indicate that the waters in these latitudes are not longitudinally homogeneous. In these regions, temperature is the primary variable affecting density. Thus, these small changes in salinity from section to section would have a negligible effect on density.

Changes in the configuration of the sigmat-isopleths can be followed in Figure 4, together with the current normal to the section determined from geostrophic computations relative to the 400-db surface. Stations on Cruise 15 were to 500 m. However, previous geostrophic computations in this region have shown that use of a deeper reference level does not significantly change the velocity profile. The isovels stop at 3½°N because geostrophic computations are not generally applicable within approximately three degrees of the equator.

There appears, in Figure 4, to be a progressive steepening in the slope of the $\sigma_t$ isopleths in the Countercurrent, with a corresponding increase in maximum current speed from 63 to 120 cm/sec. The computed transport of the Countercurrent south of 9°N increased from 18.3 × $10^6$ m$^3$/sec to 22.6 × $10^6$ m$^3$/sec from the first to the last section. The contribution of the portion of the Countercurrent north of 9°N would not greatly increase these transports.

To emphasize the time relationship of the four sections, Figure 5 shows a plot of the depth of the 70°F isotherm against latitude and time. In this region, the 70°F isotherm is normally positioned near the center of the thermocline (Fig. 1). The steepening of the slope of the thermocline in the Countercurrent is shown by the 90-mi decrease in the horizontal distance between the 150- and

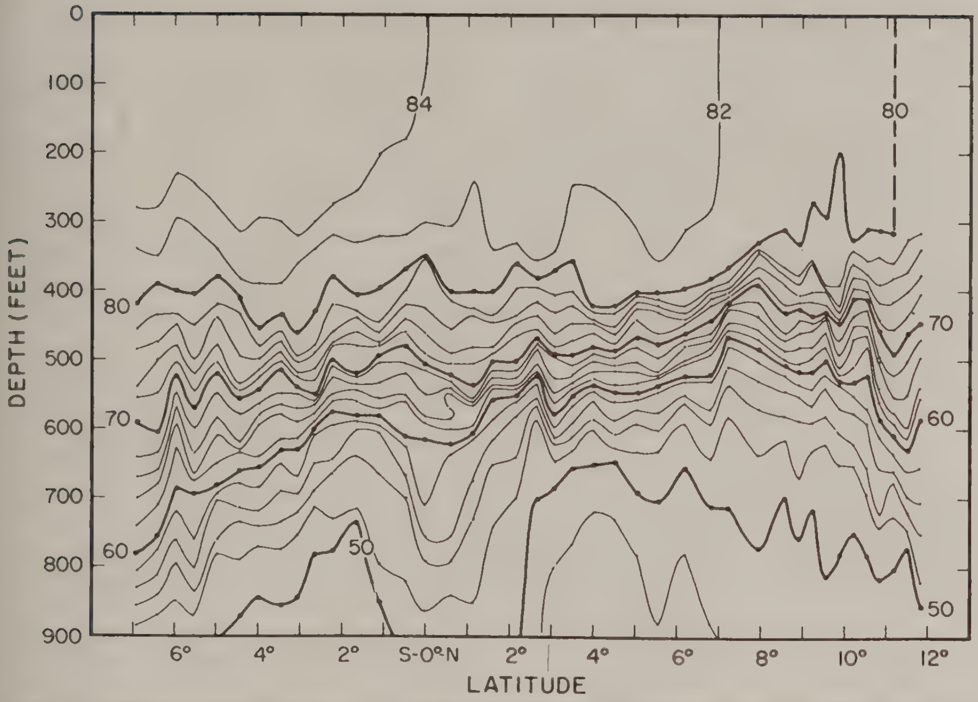

FIG. 6 – Vertical meridional distribution of temperature from *John R. Manning* Cruise 15, 170°W longitude April 28 to June 16, 1953

the 350-ft contours from Leg 1 to Leg 4. Of greater interest in Figure 5 is the evidence that some significant changes of slope in the Countercurrent took place in the relatively short time between the observations on Legs 2 and 3. While, in general, short-term variations in the distribution of mass are not reflected in corresponding velocity changes, it is probable that the changes in the Countercurrent velocity during Cruise 15 are not as regular as suggested by the results of geostrophic calculations shown in Figure 4.

Since the computed currents plotted in Figure 4 indicate only that component of the velocity normal to the sections, that is, the zonal component of flow, some of the apparent variations in speed and volume transport could be caused by north-south meandering in the Countercurrent. While these meanders cannot be ruled out, there has been no evidence of large-scale meandering during POFI's many crossings of the Countercurrent in the central Pacific, or during the recent EASTROPIC expedition, an east-west survey of the current between 160°W and 115°W longitude.

Thus, geostrophic computations show that during the period of Cruise 15 the speed of the Countercurrent doubled within four weeks. Longer-term and more pronounced changes in speed are suggested in other POFI data. On at least one previous occasion (*John R. Manning* Cruise 15) the Countercurrent, as inferred from the slope of the thermocline (Fig. 6), was nearly slack. Compare the slopes of the thermocline (10°N to 5°N) in this figure and in the more typical section shown in Figure 1. Drift of free-floating fishing gear gave further evidence that the Countercurrent was very weak (less than 15 cm/sec). It is interesting that these observations closely followed a period of one of the most striking El Niños in recent times [*Sears*, 1954].

The above discussions demonstrate that there are transients in the central Pacific Equatorial Countercurrent at least of the scale observed. It has not been possible to relate these changes to local wind conditions. Further studies, possibly ocean-wide in scope, are required to determine the causes of these fluctuations.

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(Manuscript received April 18, 1956, and, as revised, June 11, 1956; presented at the Thirty-seventh Annual Meeting, May 2, 1956; open for formal discussion until March 1, 1957.)

Deep-Sea Research, 1956, Vol. 3, pp. 178 to 188. Pergamon Press Ltd., London.

# A contribution to the natural history of the white-tip shark, *Pterolamiops longimanus* (Poey)

RICHARD H. BACKUS*, STEWART SPRINGER† and EDGAR L. ARNOLD, Jr.‡

(*Received* 12 *January*, 1956)

**Abstract**—Until recently little has been known about the common, pelagic shark, *Pterolamiops longimanus*. Data gathered during recent offshore cruises show it to be abundant and widely distributed in the warm waters of the western North Atlantic. It occurs at a wide range of salinities but withdraws from some waters when the temperature gets as low as about 21°C. It is rarely present in water shallower than about 100 fathoms. In a sample of 110 sharks few were over 250 cm in total length, although the maximum size reported in the literature is much longer. Fish and cephalopods are the most frequent food items in white-tip stomachs. White-tips are cautious, persistent, and sluggish in their behaviour. They are responsible for considerable damage to long line caught tuna in the Gulf of Mexico. Geographical sexual segregation is a feature of white-tip life history. Fragmentary data indicate that the mating and pupping season is in the late spring or early summer and that the gestation period is about one year. Females probably first mate at a length of about 200 cm, and probably bear young in alternate years thereafter. The number of pups per litter varies from 2 to 9 with a mean of about 6. Shark suckers (*Remora remora*), pilotfish (*Naucrates ductor*), dolphin (*Coryphaena hippurus*) and a copepod parasite or parasites are common associates of the white-tip.

## INTRODUCTION

RECENTLY, BIGELOW and SCHROEDER (1948), in their monograph " Sharks," wrote of the white-tip, " Astonishingly little is known of the habits of *longimanus*, considering that it is one of the members of its genus that has been recognized the longest."

That this is true is probably explained by the animal's rather strict oceanic habit which makes it largely unavailable to seaside naturalists. However, with an increasing number of offshore surveys it is becoming better known. MATHER and DAY (1954) found it to be widespread, abundant, and the commonest pelagic shark in the warm parts of the North Atlantic. BULLIS (1955) regards this shark as responsible for most of the damage to long line-caught tuna in the Gulf of Mexico, although it was quite unknown from that area prior to an investigation of offshore waters begun in 1950 by the U.S. Fish and Wildlife Service.

Although BIGELOW and SCHROEDER (1948) also assume the white-tip to be common in the Mediterranean on the basis of statements by MÜLLER and HENLE (1841), GÜNTHER (1870), DODERLEIN (1881), and others, TORTONESE (1951) states that its presence there is not now demonstrable by museum specimens or other verifiable records. MAUL (1955) records the species from Madeira.

The authors have been gathering data on the Atlantic white-tip shark independently during the course of other work for the past few years. BACKUS has made observations from the Research Vessels *Atlantis* and *Bear* of the Woods Hole Oceanographic

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† U.S. Fish and Wildlife Service, Washington, D.C.

‡ U.S. Fish and Wildlife Service, Galveston, Texas.

Institution, and Springer has made observations from the Exploratory Fishing Vessel *Oregon* of the U.S. Fish and Wildlife Service. Arnold has furnished records of captures made from the U.S.F.W.S. Vessel *Alaska* and from recent cruises of the *Oregon*. In many cases, in the absence of the authors, fishing was done and records kept by co-workers or crew members of the above vessels. Furthermore, we have used records furnished by W. W. Anderson of captures made from the U.S.F.W.S. Research Vessel *Theodore N. Gill*, and Mr. William C. Schroeder of the Woods Hole Oceanographic Institution has given us records of captures from *Caryn* and *Cap'n Bill II*. Because of this diversity of sources, our data vary in their completeness and do not show the geographic distribution of the fishing effort which produced them.

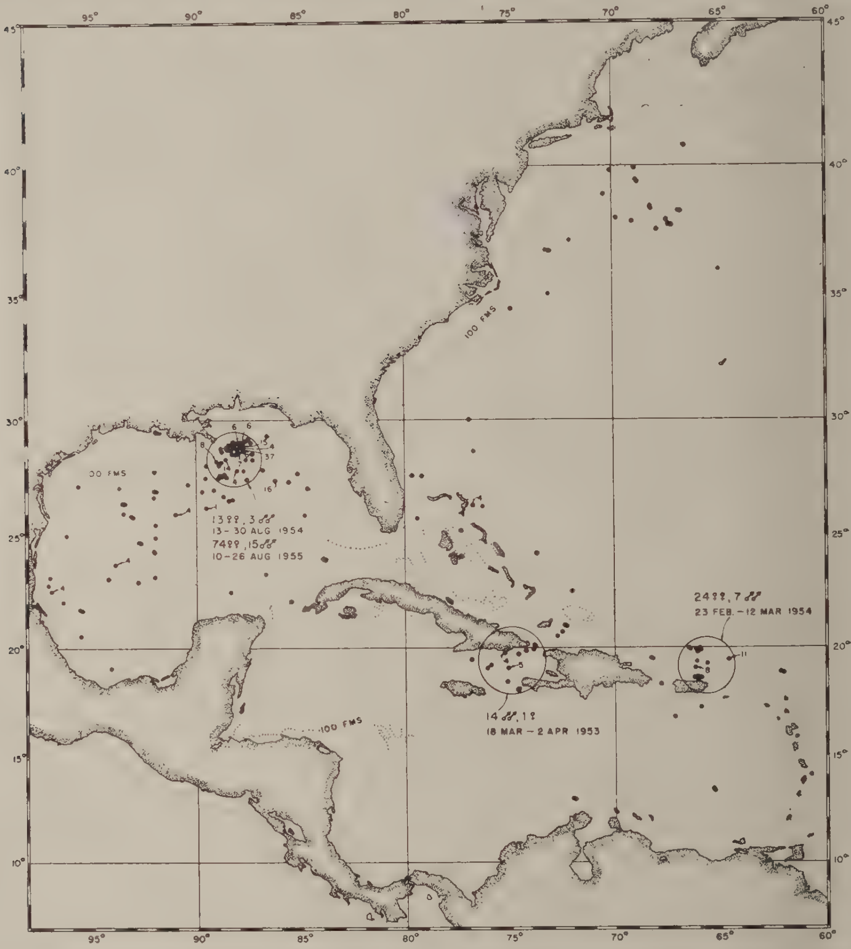
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Fig. 2. Distribution of white-tip shark captures. The scanty literature records are omitted. Three areas in which sexual segregation has been noted are encircled. All captures save one are outside the 100-fathom curve.

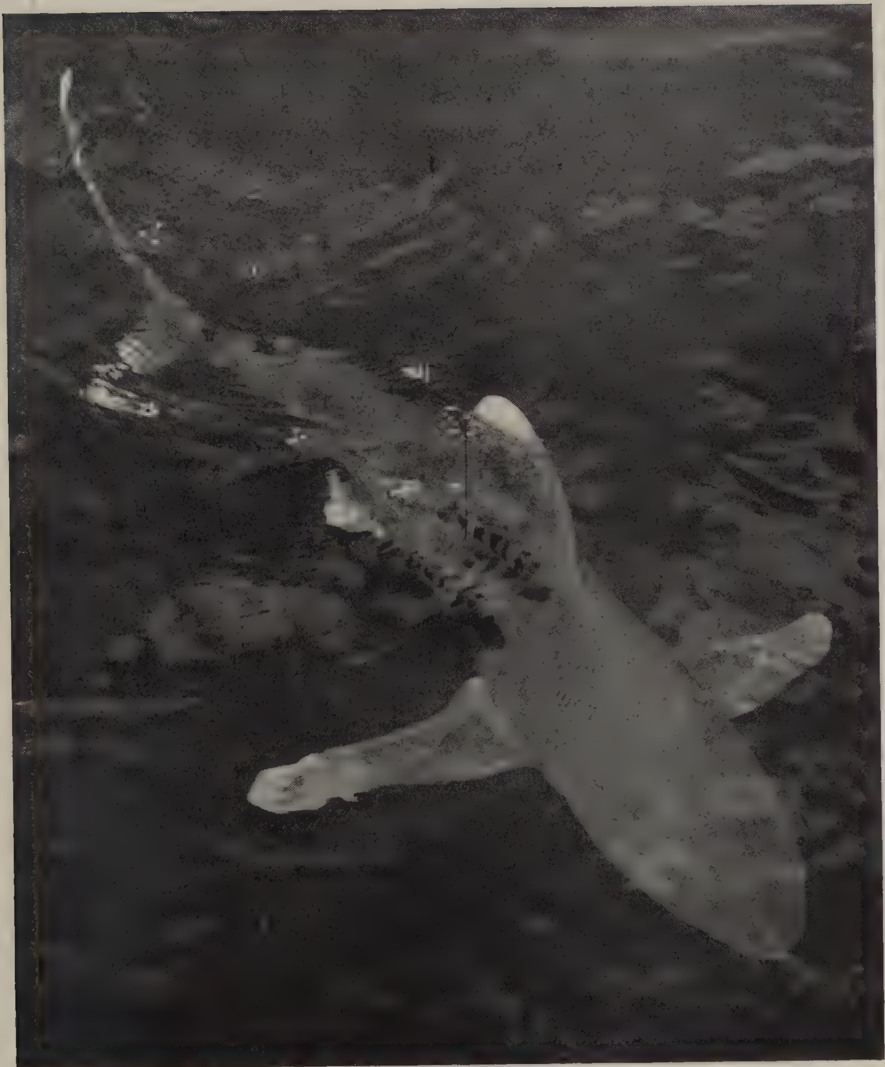

Fig. 1. White-tip shark (*Pterolamiops longimanus*) with two pilotfish, photographed at sea by JAN HAHN, Woods Hole Oceanographic Institution.

Our records (Fig. 2) are mostly from hand line and long line captures near the surface, but some sight records are included. Statements concerning the absence of white-tips from inshore waters and from parts of the Straits of Florida are largely made on the basis of SPRINGER'S observations during commercial shark fishing operations on the continental shelf off Florida, in the Gulf of Mexico, off the Caribbean coasts near Nicaragua and Trinidad, and off the coasts of the Guianas between 1930 and 1949.

## NOMENCLATURE

The species included in *Carcharhinus* as previously understood (see BIGELOW and SCHROEDER, 1948) were divided into three genera in a recent revision of western-Atlantic Carcharhinidae (SPRINGER, 1950a ; 1951). A primary division of these sharks was based on the presence or absence of a median, longitudinal ridge in the skin between the first and second dorsal fins. *Carcharhinus* was retained for the smooth-backed members of the older genus. The ridge-backed members were divided between *Eulamia* (with acuminate first dorsal and pectorals) and *Pterolamiops* (with broadly rounded first dorsal and pectorals). The white-tip belongs to the latter genus in this classification.

In general, though observations have not covered all species, the large ridge-back, carcharhinid sharks in the western North Atlantic are oceanic, although they may at times range into coastal waters. Conversely, western North Atlantic observations from off the shelf are lacking for any smooth-backed carcharhinids excepting two doubtful observations of *Negaprion brevirostris* (Poey). The smooth-backed species of the genus *Carcharhinus* (as restricted by SPRINGER) are shallow water species particularly abundant near mouths of large rivers. *Eulamia* contains sharks of the reefs, the outer parts of the continental shelf, and, to some extent, the high seas. *Pterolamiops* is strictly pelagic.

Field identification of the white-tip shark is easily made because it is the only large carcharhinid having a high first dorsal that is broadly and evenly rounded at its apex. The very large pectorals with broadly rounded tips are also distinctive. The middorsal ridge is readily observed in freshly caught late embryos, young, and adults, but its existence in preserved ones cannot always be demonstrated. Usually the pectorals and the first dorsal are tipped with white, but the amount of white showing is variable and its complete absence is fairly common. Black tips on the pelvics, second dorsal, anal, and lower caudal characterized a young example collected off Tampico, Mexico. This distribution of black spots is shown in MAUL'S figure (1955, Fig. 9). The black spots, if regularly present in young white-tips, tend to disappear in preserved specimens, and become obscure in living white-tips as they approach maturity. The pectorals and first dorsal fin of the Tampico specimen were not white-tipped, although the first dorsal was somewhat lighter near the tip.

## DISTRIBUTION

BIGELOW and SCHROEDER (1948) suggested that the physical factor which primarily determines the distribution of this " . . . more strictly pelagic . . . " shark is salinity. Data available to them indicated that this animal was not found at salinities lower than about 35·5‰. They concluded that its offshore habit was at least in part caused by an avoidance of low salinities. Thus they explained the absence of the white-tip along the east coast of the United States in summer where it might be expected on the basis of temperature alone.

Our records show that the white-tip occurs over a wide range of salinities. In 29°N, 88°W, for instance, where we have many records for July and August, the salinity may be lower than 28‰ (Dept. of Oceanography, A & M College of Texas, 1954). Furthermore, on the basis of salinity we cannot explain the absence of the white-tip from the northern and western side of the Gulf Stream in its passage through the Straits of Florida and northward along the Florida coast past the Bahamas (where the salinity is high). The area from Key West to off Palm Beach, Florida, was fished extensively by commercial shark fishermen from 1935 to 1949 using bottom set lines. Although the white-tip is apparently not available to bottom lines, regular trails of offshore fishing using floating lines were carried on. The only record of capture of a white-tip by a commercial boat was from the eastern side of the Gulf Stream near Bimini. We have caught the white-tip from early June to late October north of Cape Hatteras and west of the Gulf Stream. While we have not fished much in this region during the cold season, it seems probable that the white-tip withdraws to the south and east at this time. We have noted a withdrawl from the northern Gulf of Mexico when the waters there were cool. This may begin as early as December. In 1955 the most noticeable change in the shark fauna here occurred about 1 February. Sea surface temperatures at this time in the northern Gulf (over water deeper than 100 fathoms) are in the neighbourhood of 70°F (FUGLISTER, 1947). The withdrawal of the white-tip is accompanied by an offshore movement of some of the larger inshore sharks, notably *Galeocerdo cuvier* (Lesueur) the tiger shark, and several species of *Eulamia*.

Our records show that white-tips are ordinarily present in the surface waters where depths exceed 100 fathoms, but that they occasionally move into the adjacent shallower water. We suggest that the controlling factor is competition for food with other species of sharks which places the relatively slow-moving white-tip at a disadvantage.

The northernmost record for the white-tip in the western Atlantic, so far as we know, is for one taken in 40° 43′N, 66° 39′W on 25 July, 1953 (Fig. 2). SCHUCK and CLARK (1951) report a specimen taken in 40° 40′N, 57° 02′W on 12 June, 1950.

It has been generally supposed that the blue shark (*Prionace glauca*) is the abundant, widely-distributed, warm-water, oceanic shark. While gathering our white-tip records (Fig. 2) only one blue shark (a male, 295 cm, from between Santiago and Cabo Cruz, Cuba, 19° 30′N, 76° 50′W on 25 April, 1955) was seen by us in the area of the chart south of latitude 32°N. Published records within this area as given by BIGELOW and SCHROEDER (1948) include one each for St. Thomas, Cuba, and Miami, although HOWELL-RIVERO reported it as often seen around Cuba (in a personal communication also cited by BIGELOW and SCHROEDER). MATHER and DAY (1954) reported only 2 blue sharks (as compared with about 30 white-tips) in the wide area of the southern North Atlantic for which they had observations. This raises the point as to what the source is in the North Atlantic from which male blue sharks invade the waters off our northeast coast in such abundance in the summer.

## SIZE

Very few sharks over about 250 cm or under about 150 cm in total length were caught. Since white-tips are born at a length of about 65-75 cm (BIGELOW and

SCHROEDER, 1948) it is apparent that we have not sampled a large part of the population (75-150 cm in length) (Fig. 3). It might be argued that our hooks are too large to catch the smaller sizes, but we discount this since we have caught small specimens of other species. Furthermore, it is certain that we would see the smaller sizes swimming about the vessel and nosing the baits if they were present. A young white-tip 102 cm in total length was taken near Tampico, Mexico, in late January from a long line set near the surface where the water depth was 1,200 fathoms. No long line fishing was carried on south of this point during the winter or early spring. Some other carcharhinids (e.g. *Negaprion brevirostris*, the sub-tropical lemon shark) resort to restricted nursery areas to bear young. It is possible that we would have taken more specimens in the 75-150 cm size if we had fished in the southerly parts of the species' range in the fall and early winter. The extremely rapid growth of sharks in warm-water nursery areas may well explain the absence of specimens of this size in our collections (made mostly in the spring and summer) as has been noted for *Negaprion*, (SPRINGER, 1950b).

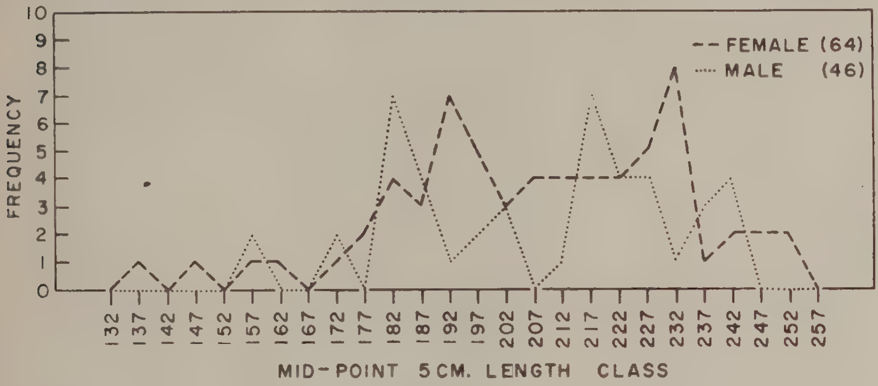

Fig. 3. Length-frequency distribution of a sample of white-tip sharks.

The longest white-tip for which BIGELOW and SCHROEDER (1948) had exact measurements was 350 cm long. This is considerably longer than our longest specimen. BIGELOW and SCHROEDER write that 12 to 13 feet (365–395 cm) is considered the maximum size, but that it is probable that an even larger size may be attained. Our data are insufficient to establish the size range of the species. However, in connection with the extremely large specimens mentioned above, a condition parallel to that found in the great hammerhead shark (*Sphyrna tudes*) is suggested. Unpublished measurements by SPRINGER of a series of adult great hammerheads show that a small percentage of the females are very much larger than the bulk of the adult breeding population. This and other observations support the possibility that some of the extreme size records for sharks persistently referred to in the literature are valid but are based on abnormal individuals, apparently always females. A false idea of the abundance of such sharks becomes established because these individuals, though solitary, eventually wander out of normal ranges into shallow water where they are easily caught. Furthermore, the capture of especially large sharks excites so much interest that they receive a disproportionate amount of attention in the literature.

A male white-tip 204 cm long weighed 60–65 kg (135–145 lb).

## FOOD

Eighty-eight white-tip stomachs have been examined. Forty-one of these contain food, although a number contained only a cephalopod beak or two. It is possible that cephalopod beaks resist digestion to a greater degree than anything else that the shark ordinarily eats. This may bias the following data : Cephalopoda – in 19 stomachs ; fish – 20 (including blackfin tuna and another scombrid fish, barracuda, look-down (*Selene*), and a white marlin about 250 cm with no evidence that it had been taken from a long line) ; garbage – 13 ; sargassum and other seaweed (probably accidental) – 4 ; unidentified animal remains – 1 ; unidentified sea bird – 1 ; Crustacea – 1.

Only a few white-tips taken on tuna long lines are included above. In general the latter had empty stomachs or contained only tuna or bait used for the tuna. One stomach contained several pounds of oil.

## BEHAVIOUR

White-tips almost always exhibit some suspicion of a baited hook. They generally make several passes at a bait before taking it. Between these passes they may swim away 50 to 100 yards. On 18 March, 1953, between captures (0915-1315) in the Windward Passage, there were about 6 white-tips around the vessel continuously. These were nosing baited hooks and would not take them although they immediately engulfed similar baits not on hooks.

Although white-tips exhibit a cautious approach to objects suspected of being edible they are remarkably persistent. A great deal of effort has been wasted by the crew of the *Oregon* in attempting to drive white-tips away from the vessel while long lines were being hauled. The long line is hauled as the vessel moves slowly forward and the line with the catch is taken over the side. A few white-tips usually gather within ten to fifty feet of the stern but swim forward and downward at the approach of a hooked tuna. The tuna come from ten or more fathoms and are not visible from the vessel when the sharks seem to detect their presence. The sharks meet the tuna more than thirty feet from the vessel and get one or more bites before the tuna can be landed. White-tips are commonly caught on the long lines and may occasionally attack spent or dying tuna on the lines before they are hauled. However, more of the shark damage well below the surface in the Gulf of Mexico results from attacks by silk sharks (*Eulamia floridana*), mako sharks (*Isurus oxyrinchus*), and occasionally by other species of *Eulamia*. All of these sharks are numerous enough to be a serious problem for tuna fishermen. We have not seen the mako sharks at the surface except when they come up on a hook or hanging onto a tuna, but silk sharks in the Gulf of Mexico are often numerous when sets are made near the continental shelf and permit comparative observations. The silk sharks are less bold than the white-tips but are much faster. Consequently, if baits are being thrown overboard in large quantities when both species are present, the white-tips get most of the baits close to the boat but the silk sharks get all of those that sink rapidly or drift some distance from the vessel. Several times we have seen a silk shark and a white-tip of about equal size in competition for the same discarded fish. If the white-tip was close enough, it got the fish by driving the silk shark away.

The white-tip does not leap when hooked and puts up a relatively feeble resistance to capture. In this respect it is comparable to the blue shark. The silk shark shows

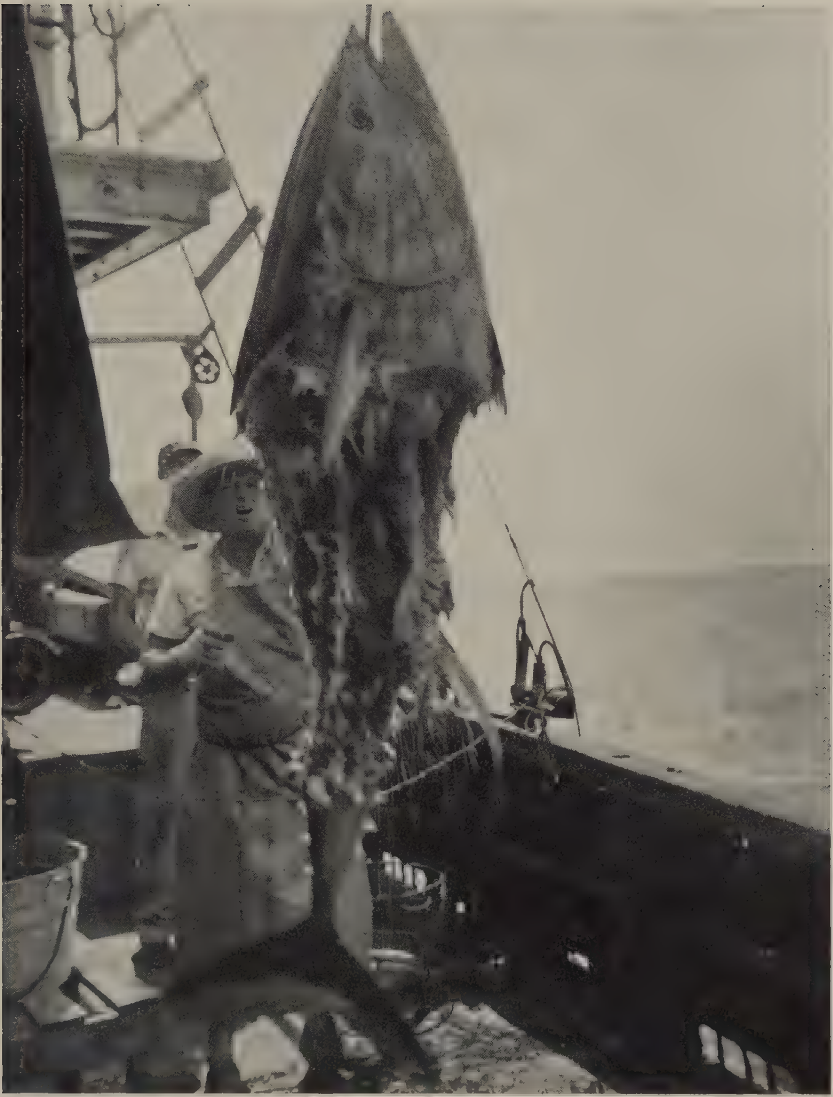

Fig. 4. Long line-caught bluefin tuna showing damage by white-tip sharks. This tuna weighed about 500 pounds before being attacked. Photo by ARNOLD.

much more speed and power than a white-tip, but once on deck the latter is more troublesome. On a few occasions white-tips have been observed to be capable of at least short bursts of speed. We have attempted to use a type of firecracker, generally known as a cherry-bomb, to drive sharks away from the vessel. The cherry-bomb was put inside a fish, the fuse lit, and the assembly tossed overboard. Due to the deliberate appraisal of the bait by the white-tip this was not often successful. The premature explosion merely caused the shark to move off a few feet, than to resume its patrol. On one occasion, however, a white-tip picked up a fish with a slow fuse and the bomb exploded in its mouth. Smoke and bits of fish blew out of its gill openings, and the shark exhibited a creditable speed in moving away.

Recently, six or seven white-tips were around the *Oregon* as long line-caught white-tips were being killed and thrown overboard. The dying specimens were never attacked although as they sank they were followed out of sight by other white-tips. Even floating livers were untouched. This reaction to livers is not typical however, as we have often used them as bait on hand lines to catch other white-tips.

When white-tips swallow objects immediately at the surface the top part of the head is lifted well out of water. On one occasion when one of us was dangling a bait in front of a shark hard by the vessel's side it made many attempts to swallow the bait before it succeeded. Such exhibitions of clumsiness make one wonder how this animal can capture live fish and cephalopods and how one (which we identified from portions of its teeth) could strike a plastic encased instrument which was being towed at night at a speed of several knots. We might say in this connection that white-tips appear to be equally active by day and night.

## SEXUAL SEGREGATION

Two types of sexual segregation are known in sharks. A " behavioural " type is exemplified by the tendency of individuals of the spiny dogfish (*Squalus acanthias*) to school with others of like size, the species sorting into aggregations of large mature females ; mature males of medium size ; immature females of medium size ; or small fish of both sexes (Ford, 1921). That a given school tends to be of one sex had been noted by earlier authors (Meyer, 1875 ; Smitt, 1895 ; and Borcea, 1906).

Ford also described what can be called " geographical " segregation in the rough-dog (*Scyliorhinus caniculus*). Several thousand specimens examined at Plymouth showed that males predominated in the winter (a peak predominance of about 65%) while females predominated in the summer (a peak of about 58%).

Geographical segregation was described by Ripley (1946) for the soupfin shark (*Galeorhinus zyopterus*). He showed that the sexes were generally unequally represented as a function of area or of depth in four California fishing areas. That this phenomenon exists in the blue shark (*Prionace glauca*) is indicated by Bigelow and Schroeder (1948) who in writing of summering fish off northeastern United States and Canada say, " . . . few, if any, females take part in this yearly incursion . . . "

We have some information showing that geographical sexual segregation is also a feature of the life history of the white-tip. In an area in the northern Gulf of Mexico whose radius is about 75 miles, 13 females and 3 males were caught during 13-30 August, 1954. A similar ratio (74 females to 15 males) was found in the same area during 10–26 August, 1955. In an area between Cuba, Hispaniola, and Jamaica with a radius of about 90 miles, 15 white-tips were caught during 18 March – 2 April, 1953, 14 being males. In an area of about 80 miles in radius north and east of Puerto Rico 24 females and 7 males were caught during 23 February – 12 March, 1954 (see Fig. 2).

The geographical segregation phenomenon is not understood, but may be related to the existence of favoured pupping areas in which females tend to congregate seasonally.

## REPRODUCTION

The fragmentary data available (Table 1, Fig. 5), suggest that both mating and pupping take place in the late spring or early summer and that the gestation period is about one year.

*Table* 1.

| *Locality* | *Date* | *Number of litters* | *Total number of specimens* | *Approx. mean total length* | *Authority* |
|---|---|---|---|---|---|
| Northeastern Gulf of Mexico | 13 Aug., 1951 | 1 | 7 | 150 | BACKUS, SPRINGER and ARNOLD |
| Northern Gulf of Mexico | 14–16 Aug., 1951 | 2 | 16 | 65 | ,, |
| Northern Gulf of Mexico | 26–28 Aug., 1951 | 2 | 14 | 160 | ,, |
| 38° 25′N, 68° 07′W | 24 Sept., 1955 | 1 | 4 | 240 | ,, |
| Central Gulf of Mexico | 15 Dec., 1954 | 1 | 4 | 410 | ,, |
| Northern Gulf of Mexico | 13 Jan., 1955 | 1 | 4 | 465 | ,, |
| North of Puerto Rico | 23 Feb.–1 Mar., 1954 | 6 | 33 | 440 | ,, |
| Trinidad Islet (20°S, 29°W) | 8 April, 1913 | 1 | 1 | 670 | NICHOLS and MURPHY (1914) |
| 28° 40′N, 78° 46′W | 3 May, 1886 | 1 | 5 | 520 | U.S. National Museum (courtesy L. P. SCHULTZ) |

We have noted two conditions of the oviducts in females without pups. In some females (generally the smaller ones) the oviducts are approximately circular in cross-section and about 10–15 mm in diameter. The oviducts are hard and the lumen is not very evident. The oviduct is held close to the dorsal wall of the body cavity by a narrow mesentery. Females in this category, which we presume are nulliparous, measured 173, 180, 189, 192, 198 and 198 cm ($\bar{x} = 188{\cdot}3$) during 23 February – 23 March, 1954 (the only period during which we have made this type of observation).

In other females (generally the larger ones) the oviducts are flattened and measure about 30–40 mm from edge to edge. They are soft and limp, and the lumen is conspicuous. The oviduct lies loosely in the body cavity suspended by a wide mesentery. We presume these females to have previously borne young. During

23 February – 23 March, 1954, such females measured 192, 209, 215, 216, 221, 230, 232, and 246 cm ($\bar{x} = 221{\cdot}2$).

Females with pups measured during this same period were 189, 205, 207, 212, 225, and 241 cm ($\bar{x} = 213{\cdot}2$).

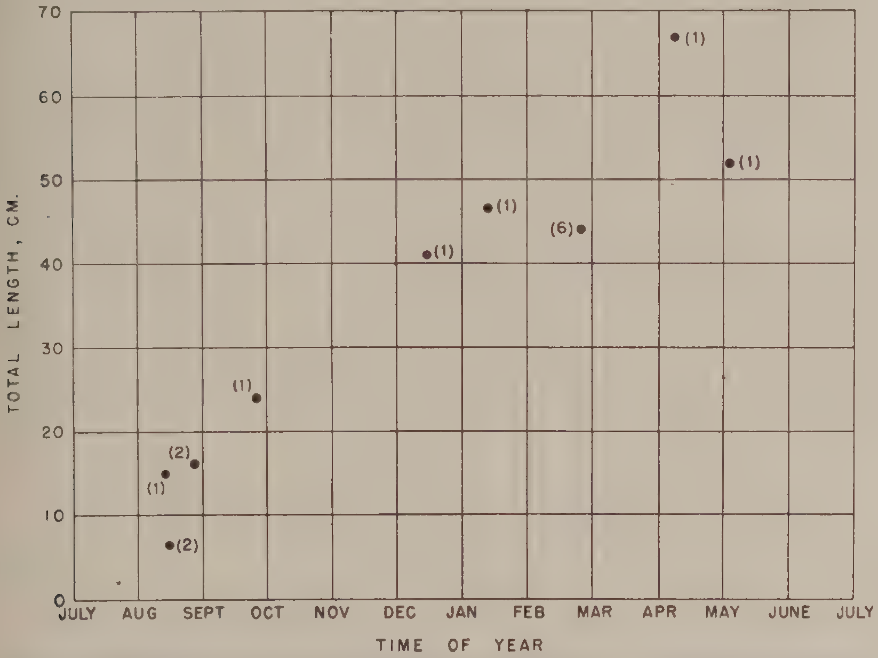

Fig. 5. Relationship between average length of embryos and time of year. The number in parentheses by each point indicates the number of litters used in arriving at the mean length.

Our original interpretation of the stretched oviducts was that females possessing them had recently dropped young. But since pregnant females at the same time were all carrying pups much smaller than the known maximum size, we no longer favour this notion. Moreover, females with stretched oviducts had large ovarian eggs measuring 25–45 mm in diameter (except one with very small eggs), while pup-bearing females had much smaller eggs (the largest being 10–15 mm in diameter). This suggests the possibility that females with stretched oviducts at this season are those that bore young the previous summer and that will mate again in the coming summer. We presume that pregnant females (with small eggs) would not mate during the coming summer.

The nulliparous females were of such a size that they could be expected to mate during the coming summer. Some of them bore ovarian eggs 10–15 mm in diameter, but some had very small eggs. None had eggs comparable in size to those of the larger non-pregnant females.

There is a good correlation (Fig. 6) between the length of the mother and the number of pups she bears ; the greater the length, the greater the number of pups. The number of pups per litter in our series (15 litters) varies from 2 to 9 with an average of about 6.

In one litter of 9, 8 embryos measured from 495 mm to 595 mm, while the ninth measured only 240 mm. It was noted that the pseudoplacental connection was lacking between the small specimen and the mother.

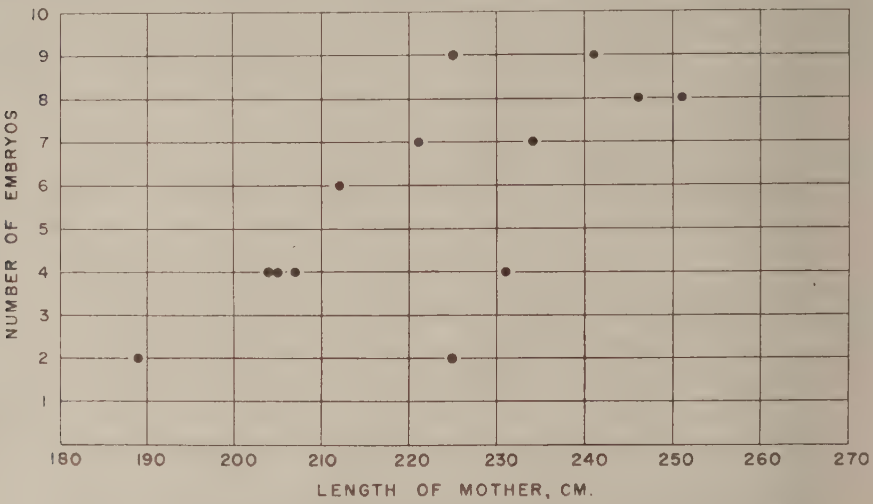

Fig. 6. Relationship between length of mother and the number of young she bears.

## ANIMAL ASSOCIATES

One to 3 shark suckers (*Remora remora*) are generally attached to white-tips. They adhere so persistently that they are taken with the shark unless scraped off as the latter is pulled in over the rail. We have frequently seen them adjust their position on the shark with a rapid wriggling motion while the latter was being boated.

Often 1 or 2 pilotfish, *Naucrates ductor*, are in company with the white-tip (see Fig. 1). They generally swim a little above and behind the shark's first dorsal fin. They swim nervously back and forth near the shark after the latter has been hooked.

On several occasions we have seen one or several dolphins (*Coryphaena hippurus*) (8-10 on one occasion) swimming with the shark. They are generally to the rear or one side of the shark and by no means follow his movements as closely as the shark pilot. SCHUCK and CLARK (1951) in reporting their capture of a white-tip wrote that the stomach contained a dolphin, and they speculate as to the condition of the dolphin when captured. They also report the absence of dolphins in the immediate area of the capture of the shark, the implication being that dolphins avoid the latter.

A copepod parasite (or parasites) which is superficially attached to the white-tip's skin is frequent and often numerous.

**Acknowledgements**—We are indebted to Capt. W. SCOTT BRAY, First Mate A. D. COLBURN, Boatswain CARL SPEIGHT, former Radioman THOMAS LYON and many seamen of the R/V *Atlantis* for help in gathering many of the shark records.

Dr. H. B. BIGELOW, Mr. W. C. SCHROEDER, Mr. W. E. SCHEVILL, and Mr. MALCOLM S. GORDON read and helpfully criticized the manuscript.

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# Some Effects of Specific Organic Compounds on Marine Organisms

In recent years it has become increasingly recognized that sea water contains organic compounds in solution or suspension which may have definite roles in the living processes of marine organisms (*1–3*). Thus it is reasonable to expect that a number of vitamins that may affect the bioeconomy of the sea are produced naturally (*4*).

This report describes two cases of interest which have come from our studies of the organic components of sea water. These results are considered to be significant because the presence of one of the compounds has been demonstrated by chemical methods (*2*) and because the other could easily be presumed to be present. In addition, both components showed definite physiological effects on the animals used.

During the experimental work of Collier *et al.*, various organic compounds were introduced into the water supply of experimental oysters [*Crassostrea virginica* (Gmelin)] in an attempt to obtain a response similar to that caused by the natural carbohydratelike substances (*5*). Niacinamide was among these, and although it did not cause an increase in pumping rate, its effect was pronounced and quantitative in nature. The effect was easily repeated from oyster to oyster under a variety of conditions. Briefly, the maximum effect was to cause an immediate increase in the gape (openness of the valves) of the oyster and, simultaneously, a complete cessation of pumping. The niacinamide was introduced into the water-circulating system in various concentrations. Table 1 summarizes the results of a series of experiments on a single oyster. The shell movement is difficult to quantitate, but, as the pumping rate decreased, the gape of the oyster gradually increased and, simultaneously, the adductor muscle lost tonus.

Table 1. Effect of niacinamide on the pumping rate of an individual oyster.

| Niacinamide (ppm) | Reduction in pumping rate (%) |
|---|---|
| 0.42 | 52 |
| 0.83 | 83 |
| 2.08 | 99 |
| 3.33 | 99 |
| 10.00 | 100 |
| 50.00 | 100 |

These data are typical, and it can be seen that the pumping rate was inversely related to the concentration of niacinamide. Some points to be noted are (i) that the substance was not acting as an irritant in the normal sense, because an irritant normally causes an oyster to snap shut or show frenetic shell movements, as compared with the quasi-narcosis in this case; (ii) that the valves could be pressed to the closed position but would immediately return to full gape; (iii) that the maximum gape caused by the niacinamide was actually about 10 percent greater than that associated with normal maximum pumping; (iv) that, as the niacinamide was gradually removed by dilution with normal, running sea water, the activity of the oyster resumed the level prevailing before the introduction of the vitamin; and (v) that niacin caused no response in similar concentrations.

Our work on the natural organic compounds in sea water is continuing and further tests with specific organic compounds are under way.

Of a series of compounds used with barnacles, ascorbic acid caused the most clear-cut response. When the barnacles (*Balanus* sp.) were exposed to a maximum concentration of 0.014 mg of ascorbic acid per liter, they immediately initiated copulating activities. The cirri ceased beating, and the penis emerged, unrolled, and sought out nearby barnacles. The ascorbic acid was introduced at one point, and because it was carried through the remainder of the 25-gal tank by convection drifts, all of the barnacles responded in the same manner. This would indicate that they were sensitive to much less than the original concentration. The experiment could be repeated at will with the same results, and changes in *p*H that were caused by adding HCl in place of ascorbic acid did not stimulate the response. The barnacles were fully grown animals that had been reared from larvae in the aquarium where the experiments were performed. These tests were made when the barnacles were 38 days old.

For comparison, glutamate, glycogen, methionine, and inositol were also used and seemed to stimulate a more rapid beat of the cirri. Fish autolysate appeared to depress the rate. None of these caused the responses noted for ascorbic acid.

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# DDT AND FISH

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The proceedings of numerous mosquito abatement meetings in recent years have included reports pertaining to the effects of insect toxicants on fish and fish food. Most of the reports have followed a rather stereotyped pattern featuring descriptions of field experiences or statements of toxicity levels and concluding with reminders that biologists and mosquito control operators must work together to make certain that no damage is done. Other insect control groups, such as the agricultural entomologists and the forest entomologists, have also sponsored studies and reports dealing with the consequences of the use of chemicals in the field. There has likewise been a uniformity of pattern in these reports. I feel that this repetition, together with the fact that few cases of mortality to fish have been shown to result from insecticide treatments in the field, has caused us to lose our caution. There seems to exist in some quarters a feeling that our knowledge of relationships between toxicants and fish is complete. It is quite understandable that this state of mind should develop, because the record for safe insecticide application is really quite good.

I would like to refer to two recent episodes which should serve to bring us back to the realization that we really understand very little about DDT and its effects on fish.

Kerswill and Elson (1955) describe the history of DDT airplane spraying for spruce budworm control in the coniferous forests of New Brunswick in Canada. Spraying of extensive areas began in 1952, and were continued in 1953 and 1954 at the rate of 0.5 pound of DDT per acre. The 1954 treatment involved a million acres and covered about one-third of the drainage of the Mirimichi River. The Mirimichi had been under investigation by fishery biologists since 1950, so considerable information on fish populations was available for two tributaries.

Careful studies of numbers of fingerling Atlantic salmon in these streams were made before and after the 1954 spraying, as well as studies on immature salmon held in live cars in the stream. After the DDT treatment no fish-of-the-year could be found in the Northwest Mirimichi, and virtually none in the Dungervon. Yearling fish were not quite so badly affected, but in one stream two-thirds of this size group were eliminated, and five-sixths of them were killed in the other stream. Among those held in live cars, the fish held in sprayed areas or downstream from sprayed areas suffered mortalities up to 91 per cent. Mortality among fish held in an untreated stream was 2 per cent. Dead and dying fish were found up to three months after the spraying.

The number of aquatic insects in these streams was seriously reduced. Dr. F. P. Ide, the entomologist who examined the insect populations after the DDT treatment, said, "At present there is virtually no suitable insect food in the streams examined. This scarcity is likely to persist for another summer at least, after which there may be large numbers of black flies and some other forms. But the insects normally associated with these fish, such as mayflies, stoneflies, and caddis flies, will probably re-establish slowly."

DDT spraying for spruce budworm in Montana and Wyoming in 1955 was done in a routine manner over thousands of acres and in several drainages. The standard rate of application was used, and, in conformity with previous experience on almost seven million acres sprayed in the United States, no damage to fish or fish food was observed in most of the drainage basins treated. In one stream system, however, DDT apparently contributed to a situation that resulted in some damage.[1]

The Yellowstone River area received an average of 0.22 pounds per acre of DDT on treated forests inside and outside of Yellowstone National Park in early July. Studies on fish and on aquatic invertebrates before and immediately after the spraying indicated that adult cutthroat trout were not directly affected by the toxicant. However, decided reductions in the aquatic insect fauna were evident in the sprayed areas a week after the treatment.

Reports from fishermen along the Yellowstone River downstream from the sprayed area began in October, and in November the complaints about dead and dying fish attracted considerable attention. Investigation by biologists revealed that there were indeed numbers of dead whitefish, suckers, and trout in the Yellowstone River, and that the affected section extended 100 miles downstream from the sprayed area. Studies on aquatic insects in the stream indicate a tremendous reduction in numbers both in and out of the sprayed section. The condition of the dying fish suggest that starvation may have been involved in the mortality on the Yellowstone.

The occurrences on the Mirimichi and on the Yellowstone should cause us to take stock of what we know about DDT and other insecticides. We know that the chlorinated hydrocarbons will kill fish and fish food, but we do not know under what conditions morbidity or mortality are caused. The treatments on these two large drainages were conducted according to standard practices, after experience elsewhere had indicated that these practices were safe. It is obvious now that we must learn more about the actions of toxicants under all physical and chemical conditions so that predictions of the consequences can be made with confidence. We shall then be in a position to use cooperative effort in insect control and achieve optimum results.

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# SOME MIGRATION PATTERNS IN CUTTHROAT TROUT

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THE migratory movements of several species of salmonid fishes have been investigated by biologists, and the literature suggests that patterns of migration are relatively constant in the salmons, trouts, and charrs. The cutthroat trout, *Salmo clarki* Richardson, has been almost completely neglected in reports on this subject. The present paper explains how migration in the cutthroat in Yellowstone Lake differs from the usual migration habits reported for the salmonids.

The Fish and Wildlife Service has studied the stream biology of the Yellowstone cutthroat, *Salmo clarki lewisi,* in Arnica Creek, a tributary of Yellowstone Lake in Yellowstone National Park, Wyoming. During the 1951 investigations, measurements and counts were made on some features of the migrations of spawners ascending Arnica Creek to spawn, and of immature trout moving downstream to the lake after having hatched from the gravels of the stream. An upstream-downstream fish trap was a permanent installation in the stream, so that all fish moving up- or downstream were trapped for processing by biologists. The trap was regularly serviced from early May to late September, so detailed information on the entire spawning population and most of the lakeward-migrating fry and fingerlings was obtained. Accessory physical data were obtained on a daily schedule.

### Diurnal Movements of Migrating Adults

Morning and evening counts of adult cutthroat in the upstream spawning migration were made in the Arnica Creek trap throughout the spawning season. Fish that entered the trap between 7 - 8 a.m. and 6 p.m. were tabulated as moving in the daytime. Adults moving at other times were listed as migrating at night. The time involved in the daytime movement was 10-11 hours, while the night migration encompassed 13-14 hours. The tabulation from May 20 to August 12, a period of 85 days, shows that many more fish moved upstream at night than in the daytime. The number of fish counted for day and night in three-day increments, is plotted in figure 1. On only 10 days of the 85 did more fish enter the trap in the daytime than at night, and eight

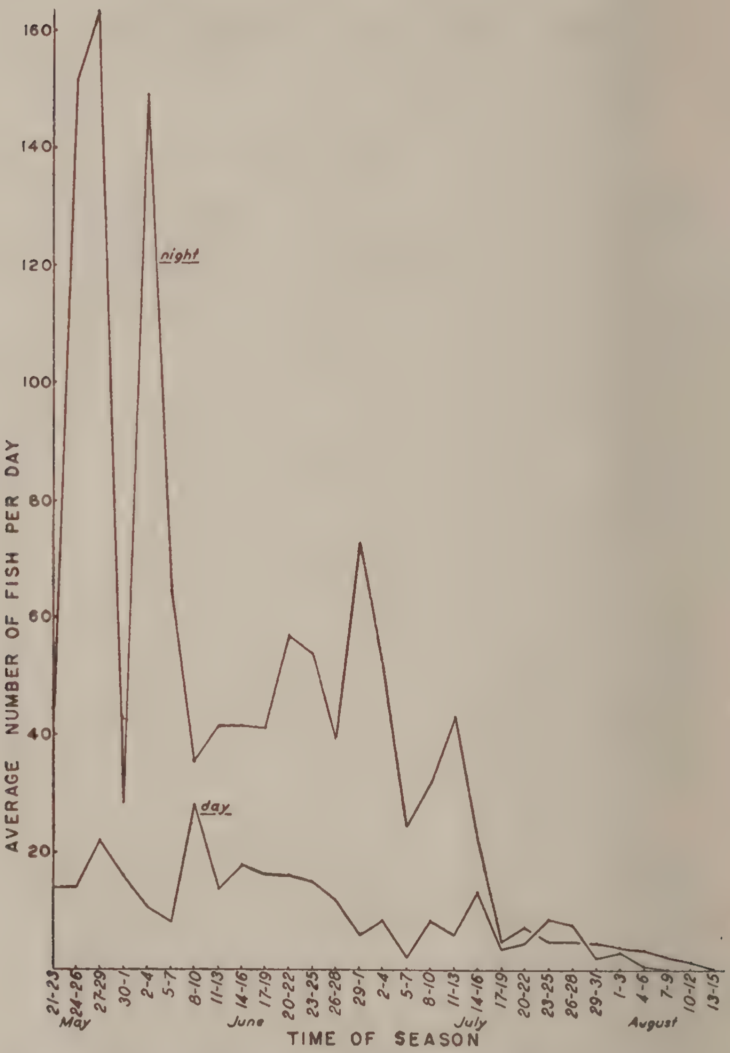

Fig. 1. *Average daily numbers of adult cutthroat entering Arnica Creek trap at night and in the daytime, from May 21 to August 15, 1951*

of these were after July 15, when the run could be considered as essentially terminated. Temporary temperature conditions may account for this divergence in the pattern. The total number of adults counted upstream during the season was 4,485. Of these, 3,647, or 81 percent, moved at night, and 838, or 19 percent, migrated during the day. The diurnal differences in migration numbers became smaller as the season progressed and virtually disappeared after the middle of July.

Data for the years since 1951 indicated that the 1951 pattern was typical for all years of observation.

Studies on salmonid fishes in other areas indicate that trout and salmon adults usually migrate upstream in the daytime. Neave (1943) showed that silver and king salmon in the Cowichan River in British Columbia migrated chiefly during daylight hours. Chapman (1941) reported on steelhead, king salmon, and red salmon at Rock Island Dam on the Columbia River, where red salmon in one ladder moved mostly in the early morning and those in the middle ladder moved predominantly in the late afternoon. King salmon and steelhead migrated heavily through the middle of the day. Shapovalov and Taft (1954) reported silver salmon and steelhead trout moved from the ocean into Waddell and Scott Creeks in California mostly in the daytime. Gustafson (1951) found that *Salmo trutta* moved into the fish ladder on the Damman River in Sweden chiefly in the daytime, with 42 percent of the fish moving between 12 noon and 4 p.m. Irving (1955) said of the cutthroat of Henrys Lake, Idaho, "That portion of the run which preceded the break-up of the ice cover on the lake migrated primarily during the afternoon periods of the warmer days. After the ice cover left the lake, the maturing trout migrated into the trap night and day." This is the only account seen in which night migrations are mentioned.

Biologists have attempted to correlate times of migration with factors of the environment. These correlations have not been completely established, but some observations seem to support the controlling influence of certain elements. Temperature seems to control some aspects of migration, as in the case at Arnica Creek in the period May 30 to June 1. There was a sharp reduction in numbers for the night migration (figure 1). The temperature record shows that abnormally low water temperatures occurred on May 31 and June 1, descending to 32° F., and resulting in an immediate cessation of the migration.

A clue to the unusual nocturnal migrations in Arnica Creek comes from Huntsman's (1945) article on freshets and fish. Huntsman studied Atlantic salmon on Cape Breton, Canada, finding that this species responds to freshets by ascent of streams, and that ascent occurs chiefly as the freshet is subsiding. Freshets normally occur daily in Arnica Creek during most of the spawning season. There is a fairly regular diurnal fluctuation in stream discharge which seems to be a function of the action of air temperatures on melting snows. At the onset of spawning in the streams tributary to Yellowstone Lake, in May, the several drainages are blanketed by a cover of snow. During the morning hours, stream flow is at a minimum for the day. When rising air temperatures prevail in the late morning, the snow begins to melt but does not yet contribute to the streams. Mid-afternoon air temperatures accelerate the snow melting process, and the streams begin to rise. The peak discharge of streams occurs in the evening, some hours after air temperatures have fallen. The day's accumulation of water normally does not reach the main stem of the spawning stream until darkness, and troubles with flooding of fish traps may occur from 10 p.m. to 12 midnight. By mid-July snows have usually melted, and this pattern of stream flow gives way to the more steady discharge characteristic of spring-fed streams.

It seems plausible that the evening peaks of water flow in these streams stimulate the upstream migration of cutthroat adults, perhaps as the freshet is subsiding, as Huntsman observed in Atlantic salmon. This could explain why nocturnal migration is characteristic of the main part of the spawning runs, while populations at lower altitudes and in streams with different discharge patterns move in the daytime. When the steady pattern of stream flow begins in the middle of July, the nocturnal migration pattern of the cutthroat spawners changes, as seen in figure 1.

### Diurnal Movements of Migrating Immatures

Morning and evening trap counts at Arnica Creek in 1951 showed that cutthroat fry and small fingerlings engaged in nocturnal movements in their downstream migrations. Although some daytime movement took place, table 1 shows that 88.3 percent of the fry entering the trap from August 1 to 15 did so during the night time. Counts for other times are not as accurate as these, but the same trend persists.

Nocturnal movement of fry is better supported in the literature than is this habit for adults. Authors have described it for species of Pacific salmon and for some trouts. As with Arnica Creek adults, the preponderance of night migrations of cutthroat fry is greatest during the heaviest period of the run. As the

Table 1. *Numbers of cutthroat fry entering the Arnica Creek trap on their lakeward migrations, August 1 to 15, 1951*

| Date | Number migrating in daytime | Number migrating at night |
|---|---|---|
| August 1 | 16 | 198 |
| 2 | 46 | 322 |
| 3 | 116 | 162 |
| 4 | 55 | 258 |
| 5 | 16 | 161 |
| 6 | 65 | 903 |
| 7 | 88 | 632 |
| 8 | 74 | 611 |
| 9 | 32 | 53 |
| 10 | 70 | 967 |
| 11 | 19 | 54 |
| 12 | 0 | 236 |
| 13 | 4 | 82 |
| 14 | 11 | 59 |
| 15 | 19 | 66 |
| Totals | 631 | 4,764 |

season progresses, daytime movement of fry increases in proportion to night migrations.

Views have been advanced to account for these nocturnal migrations of fry, and possibly the most plausible explanation is that of White and Huntsman (1938). These authors conclude that a negative phototropism is responsible for nocturnal descent of trout fry in a stream.

Measurements in more recent years indicate that the 1951 observations were representative of the normal migration pattern.

## Summary

Migrating cutthroat trout spawners in Arnica Creek, Yellowstone National Park, were counted day and night, in 1951. Five-sixths of the total count for the season entered the stream between 6 p.m. and 7-8 a.m. This nocturnal migration is unusual for salmonid spawners, and is explained by the response of spawners to evening freshets that occur during the season of melting snows.

Lakeward-migrating cutthroat fry in the same stream were counted, and it was found that 88 percent of the total count from August 1 to 15 moved downstream at night. Nocturnal movements of fry have been observed in other trouts and in salmon, and have been attributed to a negative phototropism.

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# SPAWNING AND EGG PRODUCTION OF OYSTERS AND CLAMS

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Many aspects of the physiology of reproduction of oysters and clams have been investigated, and most investigators have been impressed by the great number of eggs produced by these animals. Although the number of eggs released, by individual females during a season, is an important factor in determining what constitutes a sufficient breeding stock, this number had never been determined experimentally, and estimates of the number have varied widely.

Brooks (1880) was the first to estimate the number of eggs produced by a female American oyster, *Crassostrea virginica*. His estimate was based on the volume of eggs washed out of ripe females and the calculated volume of a single egg. From the volume calculation Brooks' figures gave 18,750,000 to 125,000,000 eggs, but he believed that as much as 50 per cent should be allowed for foreign matter washed out with the eggs. His estimate, therefore, was nine million eggs per female but states that "an unusually large oyster" gave an estimated 60 million eggs. Churchill (1920) estimated over 16 million eggs and Nelson (1921) estimated 16 to 60 million eggs per female. Galtsoff (1930) observed that individual oysters may release 15 million to 114.8 million eggs at a single spawning and estimated that the maximum number released by a single female during a season may be close to half a billion. Burkenroad (1947), although offering no experimental data, apparently believed Galtsoff's estimate was some ten times too high.

The only known estimate of the number of eggs released by a female clam, *Venus mercenaria,* is that of Belding (1912) who merely states in his summary that the average number of eggs for a 2½-inch quahaug is about two million.

The experiments we are reporting were designed to determine the total number of eggs actually produced by individual oysters and clams under experimental conditions, and to find whether there was any correlation between the size of the individual and the number of eggs produced. We also hoped to find whether this total was affected by the time interval between spawnings, or by the number of times the female spawned.

For our first experiment, 75 oysters were brought into the laboratory on December 30, 1954, and placed in conditioning trays of running sea water at 10.0°–13.0° C. The temperature of the sea water in the trays was raised to 20.0°–21.0° C. on the 31st of December. The clams were obtained on January 5, 1955 and 75 individuals were placed in running sea water at 18.0°–19.0° C. (Loosanoff and Davis, 1950).

In mid-January, after approximately two weeks at conditioning temperature, we started spawning 25 of the clams and 25 of the oysters at three-day intervals. A second group of 25 oysters was spawned at five-day intervals and a third group of 25, at intervals of seven days. The second group of 25 clams was spawned at intervals of seven days and the third group of 25 clams, at intervals of 14 days. To determine the number of eggs produced by each female, each individual was placed in a separate spawning dish, and the shells of the females were marked with a sex symbol and an individual number. The males were discarded as soon as they were identified. The number of eggs released by each female was determined and recorded each time she spawned (Tables I through VI).

The spawning records of the oysters subjected to spawning stimuli every three days are shown in Table I. Within this group the total number of eggs per female ranged from 7.8 million to 59.9 million, while the number released at a single spawning ranged up to 28.3 million. The number of spawnings per female ranged from two to 16. Only number 14 failed to spawn on two or more consecutive three-day trials, and number 10 spawned repeatedly at three-day intervals. The highest number of eggs (59.9 million) was produced by a female that spawned nine times. The female that spawned 16 times ranked second, and a female that spawned only five times ranked third. The lowest total number of eggs was released by a female that spawned seven times. Tables II and III show similar data for the groups spawned at five- and seven-day intervals.

TABLE I

*Number of eggs (in millions) released by individual female oysters induced to spawn at 3-day intervals*

| Days after initial spawning | Female number | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
| 0 | 9.0 | 2.0 | 6.8 | 7.9 | 6.3 | 0.4 | <0.1 | — | — | — | — | — | — | — |
| 3 | 1.0 | 0.8 | — | 3.8 | 8.7 | 0.5 | 2.0 | 0.9 | — | — | — | — | — | — |
| 6 | 3.1 | 8.4 | — | — | — | — | 6.8 | 2.9 | <0.1 | <0.1 | — | — | — | — |
| 9 | — | — | 0.4 | 6.9 | 16.3 | 2.5 | 3.3 | 1.3 | — | 0.1 | 1.8 | 3.3 | — | — |
| 12 | 2.8 | 18.7 | — | 1.6 | — | 1.1 | 7.7 | 2.9 | — | 4.0 | 2.7 | 2.3 | — | — |
| 15 | — | — | — | — | — | — | — | — | — | 9.2 | — | — | — | — |
| 18 | 3.9 | 5.8 | — | 4.7 | — | 1.5 | — | 2.0 | 7.1 | 6.5 | — | — | 5.6 | — |
| 21 | — | — | — | — | — | — | 9.7 | — | — | 5.6 | — | — | 1.8 | — |
| 24 | 3.8 | — | — | 3.5 | — | 1.8 | — | 3.6 | — | 4.1 | 28.3 | 16.5 | 3.6 | — |
| 27 | 0.9 | 9.6 | — | — | 10.1 | 0.02 | 7.0 | 2.3 | — | 4.0 | — | 0.1 | 3.4 | — |
| 30 | — | — | — | — | — | — | — | — | — | 2.8 | — | — | — | — |
| 33 | 4.0 | — | — | — | — | — | — | 6.6 | — | — | — | — | 6.5 | — |
| 36 | — | — | — | — | — | — | — | — | — | 5.9 | — | — | — | — |
| 39 | 1.0 | — | 1.4 | — | — | — | 3.5 | — | — | 3.6 | — | — | 3.5 | — |
| 42 | — | 9.4 | 0.5 | 3.7 | — | — | — | — | — | 2.0 | 1.2 | — | 1.7 | 8.4 |
| 45 | 0.3 | — | — | — | — | — | — | — | 27.3 | 2.4 | — | — | 1.4 | — |
| 48 | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| 51 | — | 5.2 | — | — | — | — | — | 3.2 | — | 2.7 | 9.1 | — | 1.5 | — |
| 54 | — | — | — | — | — | — | — | — | — | 0.6 | — | — | — | — |
| 57 | — | — | — | — | — | — | — | — | — | 0.2 | — | — | — | — |
| 60 | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| 63 | — | 0.03 | 1.9 | — | — | — | — | — | — | — | — | — | — | 2.1 |
| 66 | — | — | <0.1 | — | — | — | — | — | — | — | — | — | — | — |
| Total eggs | 29.8 | 59.9 | 11.0 | 32.1 | 41.4 | 7.8 | 40.0 | 25.7 | 34.4 | 53.7 | 43.1 | 22.2 | 29.0 | 10.5 |

The record for clams spawned every three days (Table IV) shows a range from 17.1 to 37.4 million in the total number of eggs released. The highest number released by any female in this group at a single spawning was 17.7 million. The num-

TABLE II

*Number of eggs (in millions) released by individual oysters induced to spawn at 5-day intervals*

| Days after initial spawning | Female number | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
| 0 | 0.7 | 0.7 | — | — | — | — | — | — | — | — | — | — | — | — |
| 5 | — | 5.2 | 0.2 | — | — | — | — | — | — | — | — | — | — | — |
| 10 | 3.4 | — | 1.2 | 1.6 | 1.0 | 6.8 | 7.0 | 3.4 | 0.1 | — | — | — | — | — |
| 15 | — | — | 5.9 | 1.1 | 2.9 | — | — | 5.5 | 9.5 | — | — | — | — | — |
| 20 | — | — | — | 3.1 | — | — | — | 8.4 | — | — | — | — | — | — |
| 25 | 10.0 | — | 11.4 | — | 5.4 | — | — | — | 10.4 | 10.1 | 7.0 | — | — | — |
| 30 | 11.8 | 32.8 | 10.6 | 9.1 | 1.9 | 15.5 | 12.6 | 8.4 | 6.6 | — | — | 2.6 | 35.6 | 9.3 |
| 35 | — | — | — | 3.0 | 0.6 | — | — | 1.7 | — | 9.2 | — | — | — | — |
| 40 | 7.4 | — | 16.1 | 1.8 | 0.1 | — | — | — | — | — | — | — | — | — |
| 45 | 1.5 | 8.9 | — | — | — | — | 0.7 | — | 1.2 | — | — | — | 11.0 | — |
| 50 | — | — | 1.2 | — | — | — | — | 0.3 | — | 4.3 | — | — | — | — |
| 55 | 6.0 | 4.2 | — | — | — | 10.7 | — | — | 0.3 | — | — | 9.1 | — | 10.8 |
| 60 | — | — | — | — | — | 2.3 | — | — | 0.2 | — | — | 0.8 | — | — |
| 65 | 0.9 | 0.4 | <0.1 | — | — | — | — | — | — | 0.2 | — | <0.1 | — | — |
| 70 | 0.2 | <0.1 | — | — | — | — | — | — | — | — | — | — | — | — |
| 75 | 0.1 | — | — | — | — | — | — | — | — | — | — | — | — | — |
| Total eggs | 42.0 | 52.2 | 46.6 | 19.7 | 11.9 | 35.3 | 20.3 | 27.7 | 28.3 | 23.8 | 7.0 | 12.5 | 46.6 | 20.1 |

ber of spawnings per female ranged from three to 10, and the female producing the highest total number of eggs spawned seven times. Second highest total was by a female spawning nine times, and the third ranking female spawned only three times. The lowest total was by a female that spawned five times. Tables V and VI give similar data for the clams spawned at seven-day and at 14-day intervals.

The three groups of oysters, spawned at different intervals, were compared after arranging the females according to their rank in egg production (Table VII). The range in total number of eggs per female was from 10 thousand to 66.4 million.

TABLE III

*Number of eggs (in millions) released by individual oysters induced to spawn at 7-day intervals*

| Days after initial spawning | Female number | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
| 0 | 0.9 | 0.8 | 0.2 | — | — | — | — | — | — | — | — | — | — | — | — |
| 7 | 0.8 | 0.4 | — | 12.6 | — | — | — | — | — | — | — | — | — | — | — |
| 14 | 21.0 | 22.2 | 5.4 | 10.1 | 22.0 | 0.5 | 0.3 | 0.2 | 0.7 | 0.4 | — | — | — | — | — |
| 21 | — | — | — | — | — | — | 3.1 | — | — | 1.0 | 1.4 | 7.7 | 0.4 | — | — |
| 28 | 19.4 | 39.0 | — | — | 5.7 | — | — | 23.4 | 8.6 | — | 14.2 | — | — | — | — |
| 35 | 7.7 | 1.6 | 10.3 | — | — | — | 17.0 | — | 2.5 | 8.4 | 25.2 | — | 9.0 | 0.5 | — |
| 42 | 1.8 | 1.9 | 0.9 | 4.0 | — | 48.8 | — | — | — | 0.7 | 6.2 | 12.2 | 1.4 | — | 0.01 |
| 49 | 0.6 | — | — | <0.1 | — | — | — | 13.8 | — | — | — | — | — | — | — |
| 56 | — | 0.5 | — | — | 0.6 | 3.3 | — | — | — | — | 3.2 | — | — | — | — |
| 63 | <0.1 | — | — | — | <0.1 | — | — | — | — | — | 0.1 | — | — | — | — |
| 70 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| Total eggs | 52.2 | 66.4 | 16.8 | 26.7 | 28.3 | 52.6 | 20.4 | 37.4 | 11.8 | 10.5 | 50.3 | 19.9 | 10.8 | 0.5 | 0.01 |

TABLE IV

*Number of eggs (in millions) released by individual clams induced to spawn at 3-day intervals*

| Days after initial spawning | Female number | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
| 0 | 0.3 | 7.7 | 1.1 | 9.3 | 4.9 | 8.5 | 13.6 | 5.7 | 4.7 | — | — |
| 3 | — | — | — | — | — | — | — | — | — | — | — |
| 6 | — | 2.1 | — | — | — | — | 6.4 | — | — | 13.5 | — |
| 9 | 6.4 | — | — | 3.5 | 5.9 | 3.9 | — | — | 5.4 | 1.3 | 11.1 |
| 12 | — | — | — | — | — | — | — | — | — | — | — |
| 15 | 2.1 | 4.2 | 17.7 | 1.6 | 2.5 | 2.6 | 4.0 | 6.3 | 1.6 | 2.3 | 2.5 |
| 18 | 1.4 | — | — | — | — | — | — | — | 1.0 | — | — |
| 21 | 0.7 | 3.5 | — | — | 4.0 | 0.1 | — | 1.9 | — | 3.1 | — |
| 24 | 1.7 | — | 10.0 | 2.4 | — | 3.4 | — | 0.7 | — | 1.1 | 3.7 |
| 27 | 1.1 | — | — | 1.0 | 2.2 | 1.8 | 6.3 | — | — | — | 1.8 |
| 30 | 1.4 | — | — | — | — | — | — | — | — | — | — |
| 33 | — | — | — | — | 1.8 | — | — | — | — | 1.4 | — |
| 36 | — | 4.2 | — | — | — | 5.0 | — | — | 5.0 | 0.8 | 1.0 |
| 39 | — | — | — | — | — | — | — | 2.5 | — | — | — |
| 42 | — | — | — | 3.2 | 3.7 | 2.2 | 0.3 | — | — | — | — |
| 45 | — | — | — | — | 0.5 | — | — | — | — | — | — |
| 48 | — | — | — | — | — | — | — | — | — | — | — |
| 51 | 3.4 | — | — | — | — | — | — | — | — | — | — |
| 54 | — | — | — | — | 2.4 | — | 6.6 | — | — | — | — |
| 57 | — | — | — | — | — | — | — | — | — | — | — |
| 60 | — | — | — | — | 0.2 | 1.9 | — | — | — | — | — |
| 63 | — | — | — | — | — | — | — | — | — | — | — |
| 66 | — | — | — | — | — | — | — | — | 0.2 | — | — |
| 69 | — | — | — | — | — | — | 0.2 | — | — | 1.5 | — |
| Total eggs | 18.5 | 21.7 | 28.8 | 21.0 | 28.1 | 29.4 | 37.4 | 17.1 | 17.9 | 25.0 | 20.1 |

TABLE V

*Number of eggs (in millions) released by individual clams induced to spawn at 7-day intervals*

| Days after initial spawning | Female number | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
| 0 | 4.1 | 2.0 | 3.5 | 2.8 | 0.1 | — | — | — | — | — | — | — | — |
| 7 | — | — | 5.2 | — | — | 10.2 | — | — | — | — | — | — | — |
| 14 | 3.4 | 17.8 | 2.7 | 4.2 | — | 2.4 | — | — | — | — | — | — | — |
| 21 | 3.2 | 0.5 | 0.2 | 4.4 | 19.3 | 3.4 | 11.4 | 13.1 | 14.2 | 2.4 | 5.5 | — | — |
| 28 | — | 5.7 | 7.2 | 1.5 | — | — | — | 2.4 | — | — | 3.7 | 12.5 | — |
| 35 | 3.1 | 0.7 | 0.7 | 2.7 | 10.4 | 0.4 | 5.6 | 1.7 | — | 2.1 | — | — | 14.7 |
| 42 | 2.0 | 4.4 | 1.6 | 3.0 | — | — | 0.2 | — | — | — | — | — | — |
| 49 | 2.1 | 1.7 | 1.9 | 1.6 | — | 5.2 | 3.6 | 3.2 | 18.8 | — | 11.1 | 7.5 | 4.1 |
| 56 | 1.4 | 1.5 | — | 1.4 | — | 0.3 | 0.7 | 0.8 | — | 3.3 | 1.6 | — | — |
| 63 | 0.8 | 0.9 | — | 0.7 | — | — | 0.6 | 0.3 | — | — | — | — | 1.9 |
| 70 | 0.6 | 0.4 | 1.0 | — | 4.8 | — | 0.1 | 0.2 | — | 0.2 | 1.5 | — | 0.2 |
| 77 | — | — | — | — | 2.3 | — | <0.1 | — | — | <0.1 | — | — | — |
| 84 | 0.8 | 0.1 | 0.2 | 0.6 | 2.3 | — | — | — | — | — | — | — | — |
| 91 | — | — | — | — | 0.3 | — | — | — | — | — | — | — | — |
| 98 | 0.3 | — | — | — | — | — | — | — | — | — | — | — | — |
| Total eggs | 21.8 | 35.7 | 24.2 | 22.9 | 39.5 | 21.9 | 22.2 | 21.7 | 33.0 | 8.0 | 23.4 | 20.0 | 20.9 |

TABLE VI

*Number of eggs (in millions) released by individual clams induced to spawn at 14-day intervals*

| Days after initial spawning | Female number | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
| 0 | 7.0 | — | — | — | — | — | — | — | — | — | — | — | — | — |
| 14 | 2.0 | 2.8 | 21.3 | 10.0 | 3.3 | 5.5 | 20.4 | 10.5 | 6.4 | 2.7 | — | — | — | — |
| 28 | 7.7 | 7.3 | 3.3 | 3.5 | 22.4 | 8.0 | 8.8 | 8.8 | 13.3 | 18.5 | 19.8 | 24.3 | 10.5 | 0.7 |
| 42 | 3.2 | 2.7 | 3.3 | 5.4 | 4.2 | 4.0 | 4.3 | 3.2 | 4.3 | 1.1 | — | 2.7 | — | 8.7 |
| 56 | 1.4 | 0.5 | 2.6 | 1.6 | 2.2 | 1.6 | 1.3 | 2.0 | 1.5 | 8.0 | 9.3 | 9.1 | 3.4 | 1.5 |
| 70 | 0.2 | — | 0.4 | 0.3 | 1.1 | — | 1.6 | 1.2 | 0.4 | 2.9 | 0.7 | — | — | 0.1 |
| 84 | — | — | 0.3 | <0.1 | 0.3 | — | — | 0.6 | 0.3 | 1.6 | — | — | — | 0.2 |
| 98 | 0.1 | — | — | — | — | 0.2 | 0.1 | 0.2 | — | — | — | <0.1 | 1.0 | — |
| 112 | — | — | — | — | — | — | — | — | — | 0.5 | — | — | — | — |
| Total eggs | 21.6 | 13.3 | 31.2 | 20.8 | 33.5 | 19.3 | 36.5 | 26.5 | 26.2 | 35.3 | 29.8 | 36.1 | 14.9 | 11.2 |

Despite the relatively great differences between females, however, the range in all three groups is essentially similar, and the mean number of eggs per female in all three groups is approximately the same. The 14th and 15th ranking females, spawned at seven-day intervals, appear to have abnormally low totals. Except that

TABLE VII

*Total number of eggs (in millions) discharged by individual oysters spawned at 3-, 5-, or 7-day intervals, and by individual clams spawned at 3-, 7-, or 14-day intervals. In each group the females are ranked according to egg production*

| Oysters | | | | Clams | | | |
|---|---|---|---|---|---|---|---|
| Rank | 3-day spawning interval | 5-day spawning interval | 7-day spawning interval | Rank | 3-day spawning interval | 7-day spawning interval | 14-day spawning interval |
| 1 | 59.9 | 52.2 | 66.4 | 1 | 37.3 | 39.5 | 36.5 |
| 2 | 53.6 | 46.6 | 52.6 | 2 | 29.5 | 35.7 | 36.1 |
| 3 | 43.1 | 46.6 | 52.2 | 3 | 28.8 | 33.0 | 35.3 |
| 4 | 41.3 | 42.0 | 50.3 | 4 | 28.2 | 24.2 | 33.5 |
| 5 | 40.0 | 35.3 | 37.4 | 5 | 25.1 | 23.4 | 31.2 |
| 6 | 34.4 | 28.3 | 28.3 | 6 | 21.7 | 22.9 | 29.8 |
| 7 | 32.2 | 27.7 | 26.7 | 7 | 21.0 | 22.2 | 26.5 |
| 8 | 29.8 | 23.8 | 20.4 | 8 | 20.2 | 21.9 | 26.2 |
| 9 | 29.0 | 20.3 | 19.9 | 9 | 18.7 | 21.8 | 21.6 |
| 10 | 25.6 | 20.1 | 16.8 | 10 | 18.0 | 21.7 | 20.8 |
| 11 | 22.2 | 19.7 | 11.8 | 11 | 17.0 | 20.9 | 19.3 |
| 12 | 11.0 | 12.5 | 10.8 | 12 | — | 20.0 | 14.9 |
| 13 | 10.4 | 11.9 | 10.5 | 13 | — | 8.0 | 13.3 |
| 14 | 7.8 | 7.0 | .5 | 14 | — | — | 11.2 |
| 15 | — | — | .01 | 15 | — | — | — |
| Mean | 31.4 | 28.1 | 27.0 | Mean | 24.1 | 24.2 | 25.4 |
| Average per spawning | 4.4 | 5.5 | 7.2 | Average per spawning | 3.6 | 3.6 | 4.7 |
| Maximum number of eggs from one female at a single spawning 48,780,000 | | | | Maximum number of eggs from one female at a single spawning 24,300,000 | | | |

they were the last to spawn, spawned only once, and released exceptionally few eggs, there seems to be no reason to exclude them.

A similar comparison of the three groups of clams spawned at different intervals (Table VII) shows that they have even more closely comparable ranges, in the total number of eggs per female, than do oysters. The mean numbers of eggs per individual in the three groups of clams are also in closer agreement.

An analysis of variance test confirms the conclusion that varying the spawning interval from three to seven days for oysters and from three to 14 days for clams did not affect the total egg production. We believe that the similarity of variances and the agreement of means further indicate that each group represented an adequate sample of the populations used.

From Table VII we see that the maximum number of eggs released by one female at a single spawning, in either clams or oysters, may be greater than the seasonal total of many other females in the same population. We also note that the average number of eggs per spawning increases progressively, in oysters, as the interval between spawnings is increased. In clams there is no difference between the groups spawned at three- and seven-day intervals, but the average number of eggs per spawning increases slightly as the spawning interval is increased to 14 days.

If we compare clams with oysters, we find that clams are more uniform than oysters in egg production. The average number of eggs per female varied less from group to group (Table VII), and the range in total number of eggs released by different female clams was not so great. Moreover, as shown by the frequency distribution, the number of female clams producing different total numbers of eggs had a fairly normal distribution with a well-defined modal class at 20 to 24.9 million eggs per female, and the arithmetic mean number of eggs per female (24.6) falls within this class. In oysters, by contrast, the distribution was not obviously a normal one, and there is no well-defined modal class near the arithmetic mean (28.8 million). A comparison of the frequencies of individual spawnings in which different numbers of eggs were released also illustrates the greater variability of oysters since, again, the clams have a lesser range and a somewhat better-defined modal class.

The average number of spawnings per oyster decreases progressively as the interval between spawnings increases, while with clams there is little difference among the three groups. An analysis of variance confirms the conclusion that varying the spawning interval for oysters significantly affects the number of times a female oyster will spawn, with those subjected to spawning stimuli at shorter intervals spawning more frequently. The analysis also shows that varying the spawning interval from three to 14 days had no significant effect on the average number of spawnings per individual clam.

To find whether any correlation existed between the size of the female oyster and the number of eggs produced, we chose the volume of the shell cavity as our best criterion of size. The total number of eggs produced, expressed in millions, was then plotted against the volume of the shell cavity in ml. for each female (Fig. 1). In plotting, different symbols were used to differentiate the groups spawned at different intervals. The plot shows that, while the three separate correlations would differ, there is no striking reversal of trend. The data were therefore combined, and one over-all correlation was computed for all oysters in the experiment considered as a single group. The resulting correlation was reasonably good: $r$ was .54 (significant at the .01 level for 41 degrees of freedom), which means that about 30 per cent of the variation in total egg production for oysters could be attributed to the differences in size as denoted by cavity volume.

For clams a similar plot of the number of eggs in millions was made against the shell cavity volume in ml. (Fig. 2). Again, we used different symbols for the groups spawned at different intervals, but the data were combined for computing an over-all correlation. For clams, $r$ was .38 (significant at the .05 level for 36 degrees of freedom), which means that about 15 per cent of the variation in total egg production could be attributed to the differences in size of the female clams used in the experiment.

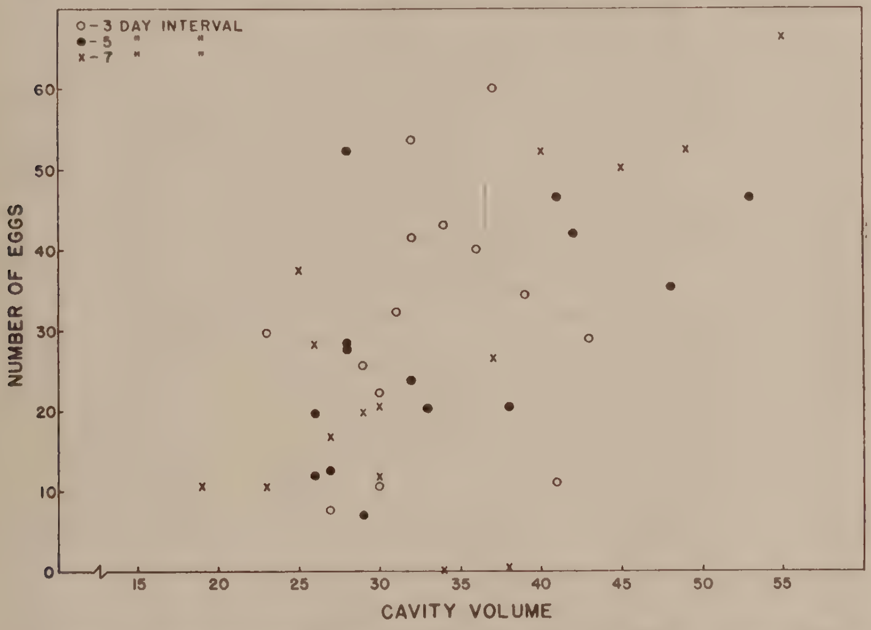

FIGURE 1. Scatter diagram showing the total number (in millions) of eggs produced, plotted against the volume (in milliliters) of the shell cavity, for each of 43 oysters. Different symbols are used to differentiate between females spawned at 3-, 5-, and 7-day intervals.

A similar test was made for a correlation between the number of times a female spawned and the total number of eggs produced. The correlation for oysters was .51 (significant at the .05 level). Thus, in general, females that have a large number of eggs to release will spawn at more frequent intervals than females having a lesser total number of eggs. For clams this correlation was only .17, or was not significantly different from zero.

It should be remembered that the oysters and clams used in this experiment were brought into the laboratory in mid-winter and that conditioning, while in trays of running sea water, does not provide optimum feeding conditions. Field observations in Long Island Sound have shown that, in some seasons, oysters that go through the winter with relatively little glycogen may increase this reserve by as much as two or three times in the spring before gonad development begins. Moreover, Loosanoff and Nomejko (1951) showed that the average gonad thickness of oysters in Long Island Sound, at the beginning of some spawning seasons, may be almost double that recorded at corresponding periods of other years. Although no measurements of gonad thickness or glycogen content were taken at the beginning of the experiment, from inspection we believe that the condition of the oysters used in these experiments was below average for oysters of Long Island Sound.

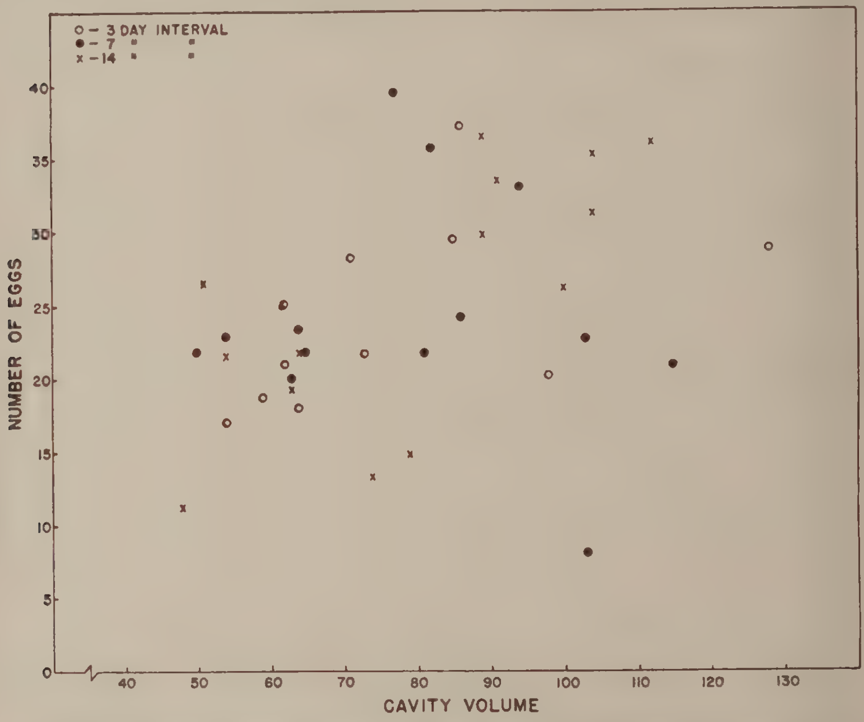

FIGURE 2. Scatter diagram showing the total number (in millions) of eggs produced, plotted against the volume (in milliliters) of the shell cavity, for each of 38 clams. Different symbols are used to differentiate between females spawned at 3-, 7-, and 14-day intervals.

A similar experiment, involving only nine females, was started on June 27, 1955 with oysters that had developed gonads under normal conditions in Long Island Sound. The total number of eggs per female ranged from 23.2 million to 85.8 million, and averaged 54.1 million eggs per female. Thus, both the average number of eggs and the maximum number per female were about 20 million higher than in the winter experiment. This was, we believe, at least in part, the result of these oysters having built up additional reserves of glycogen during the spring prior to the initiation of gonad development. Even these oysters, however, gave far fewer eggs than the ½ billion per female suggested by Galtsoff (1930).

From the present studies, and from another experiment in which 70,000,000 eggs were released by one female at a single spawning, we are in general agreement with Galtsoff (1930) on the maximum number of eggs a female oyster may release at a single spawning. Galtsoff's estimate of ½ billion eggs as about the maximum number a female oyster might release in a single season was based, in part, on the assumption that a female might release approximately 100 million eggs at each of five or six spawnings during a season. Our experiment indicates that this would be unlikely. For example, the female that gave the highest number of eggs at a single spawning, 48.8 million, spawned on only two other occasions during the experiment, and on each of these occasions released less than four million eggs. Of the 43 female oysters in the experiment, only one released in excess of 10 million eggs at as many as three spawnings.

At the conclusion of the experiment, all the females were opened and the gonad condition, presence of shell injuries, and degree of Polydora and sponge infestations were noted for each female. Statistical analysis does not reveal any effect of shell injuries, or of Polydora and sponge infestations, on the number of eggs produced by a female oyster. Histology indicated that only one female oyster still contained a few eggs, but that all the female clams still retained a few apparently mature eggs (Loosanoff, 1937).

We were surprised to find that it required about 2½ months, or approximately the duration of a normal spawning season, to completely spawn out either oysters or clams, even though they were subjected to spawning stimuli every three days. Moreover, there was almost no difference in the time required at the different spawning intervals used in this experiment.

There were marked differences, however, between clams and oysters in their behavior when subjected to chemical and thermal stimulation in the spawning dishes. Usually 80 per cent to 100 per cent of the oysters opened within 15 or 20 minutes after being placed in the spawning dishes, and normally they remained open until after spawning or until they were disturbed. Records available on 183 of the 227 spawnings of female oysters, included in the present experiment, showed how long each female was open before starting to spawn. This period ranged from one minute to 219 minutes, with an average of 34.2 minutes. Approximately 80 per cent of the spawnings occurred 30 minutes or less after the female opened, about 10 per cent of the spawnings occurred within 31 to 60 minutes, and about 10 per cent of the spawnings occurred over an hour after the female had opened and started pumping.

Clams, on the other hand, were much less predictable. A few opened almost immediately after being put in the spawning dishes, some opened only after several hours, and usually there were a few that remained closed throughout an attempted spawning. Even after opening they frequently closed again for variable lengths of time for no apparent reason. The interval between the time the clams first opened and the time they started spawning was recorded for 208 of the 235 spawnings in this experiment. This interval ranged from less than five minutes to 840 minutes, with an average of 137 minutes. Moreover, only 35 per cent of the spawnings occurred within one hour after the clams first opened. Both male and female clams have been observed to spawn for a short time, then, while still remaining open, cease spawning for periods of an hour or more, and finally spawn even more heavily than at first. Also, on several occasions both male and female clams that had been open and pumping vigorously for 30 minutes or more started spawning immediately when placed in dishes of fresh, cooler sea water.

TABLE VIII

*Spawning record of male and female oysters subjected to spawning stimuli daily for five consecutive days;* x *indicates spawning*

| Females | | | | | | Males | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Female number | Days | | | | | Male number | Days | | | | |
| | 1st | 2nd | 3rd | 4th | 5th | | 1st | 2nd | 3rd | 4th | 5th |
| 1 | x | — | x | — | — | 1 | x | x | x | x | x |
| 2 | x | — | x | — | — | 2 | x | x | x | — | x |
| 3 | x | x | x | x | — | 3 | x | x | x | — | x |
| 4 | x | — | x | x | x | 4 | x | x | x | x | — |
| 5 | x | x | x | x | x | 5 | x | x | x | — | x |
| 6 | x | — | x | x | x | 6 | x | x | x | — | — |
| 7 | x | — | x | — | x | 7 | x | x | x | x | x |
| 8 | x | x | x | x | x | 8 | x | x | x | x | x |
| 9 | x | — | — | x | — | 9 | x | x | — | — | x |
| 10 | x | — | x | x | — | 10 | x | x | x | — | x |
| 11 | x | x | x | x | x | 11 | x | x | x | x | x |
| 12 | x | x | — | — | x | 12 | x | x | x | — | x |
| 13 | x | x | x | x | — | 13 | x | x | x | — | x |
| 14 | x | — | x | x | — | 14 | x | x | x | — | x |
| 15 | x | x | x | — | — | 15 | x | x | x | — | x |
| 16 | x | x | — | — | — | 16 | x | x | x | — | x |
| 17 | x | x | — | x | — | 17 | x | x | x | — | x |
| 18 | — | x | — | — | x | 18 | x | x | x | x | x |
| 19 | — | x | — | x | — | 19 | x | x | x | x | x |
| 20 | — | — | x | — | x | 20 | x | x | x | — | x |
| 21 | — | — | x | — | — | 21 | x | x | x | x | x |
| 22 | — | — | x | x | — | 22 | x | x | x | — | x |
| 23 | — | — | x | — | — | 23 | x | x | x | x | x |
| 24 | — | — | x | — | — | 24 | x | x | x | — | x |
| | | | | | | 25 | — | x | x | x | — |
| | | | | | | 26 | — | — | x | — | — |

As noted in Table I, only one of the 14 female oysters failed to spawn two or more consecutive times when subjected to spawning stimuli every third day, and female number 10 spawned repeatedly at three-day intervals. This led us to suspect that at least some female oysters might not show the two- to five-day refractory period that Galtsoff found (1938, 1940). An experiment was therefore designed to determine whether some female oysters might spawn at more frequent intervals.

For this experiment, 50 oysters were brought into the laboratory April 4, 1955, and placed in conditioning trays at 20.0°–21.0° C. Twenty-one days later these oysters were placed in individual spawning dishes, and both chemical and thermal stimulation were used to induce spawning. Seventeen females and 24 males responded on the first day (Table VIII). After spawning, the males and females were washed separately in cold running sea water, and the shell of each was marked with the appropriate sex symbol and an individual number. All males were then returned to one conditioning tray, and all the females to another tray. Those oysters that did not spawn on this first trial were returned to a third tray. Each morning, for five consecutive days, all oysters were subjected to spawning stimuli and a record of the spawning of each individual was kept.

As shown in Table VIII, of the 24 females in the experiment, 14 spawned on two or more consecutive days, eight spawned on three or more consecutive days, five spawned on four or more consecutive days, and three spawned on each of the five days of the experiment. As was to be expected, 25 of the 26 males in the experiment spawned on three or more consecutive days, nine spawned on four or more consecutive days, and eight spawned on each of the five days of the experiment. By the fifth day, however, it was difficult to induce spawning and the spawning of both males and females was light. However, when these oysters were again subjected to spawning stimuli, after remaining undisturbed in the conditioning trays for an additional week, 22 of the 26 males and 18 of the 24 females responded, releasing approximately average amounts of spawn.

From our experiments, we believe that either there is no refractory period for female oysters, such as Galtsoff (1938, 1940) described, or it is less than 24 hours in duration. Our results suggest that both male and female oysters can spawn, upon proper stimulation, any time they have physiologically-ripe sex cells to discharge. Under constant, or closely spaced intervals of stimulation, we believe they may so deplete their supply of physiologically-ripe sex cells that they are unable to release more until additional sex cells have become physiologically mature. Some females, for example, were observed to give fairly typical spawning motions on the fourth and fifth days of the above experiment without releasing any eggs and yet, spawned normally after several additional days of conditioning.

We wish to express our thanks to the Director of Milford Laboratory, Dr. V. L. Loosanoff, who suggested this problem, for his advice throughout the experiments, and to our colleagues, Mrs. Barbara Myers, for the statistical treatment, and Mr. C. A. Nomejko, for the figures.

## SUMMARY

1. The total number of eggs released by individual female oysters, *C. virginica,* conditioned in mid-winter, ranged from 10 thousand to 66.4 million, and averaged 28.8 million.

2. Females that developed gonads under natural conditions in early summer released from 23.2 to 85.8 million eggs for an average of 54.1 million eggs per female.

3. In this experiment the highest number of eggs released by a female oyster in a single spawning was 48.8 million, but in a previous experiment one female discharged 70.0 million. Thus, some females release more eggs at a single spawning than other females do in a season.

4. There was no significant difference in the average number of eggs released in a season whether the oysters were spawned at three-, five-, or seven-day intervals.

5. Female oysters that had a large number of eggs to release tended to spawn more frequently than females that had a lesser number.

6. The average number of spawnings per female oyster decreased progressively as the interval between spawnings was increased.

7. The total number of eggs produced showed a correlation of .54 (significant at .01 level) with the size of the female oyster, as indicated by shell cavity volume.

8. No correlation could be found between the number of eggs produced and Polydora or sponge infestation, or shell injury.

9. We find no two- to five-day refractory period during which female oysters cannot be induced to spawn, as reported by earlier investigators.

10. The total number of eggs released by individual female clams, *V. mercenaria,* ranged from eight million to 39.5 million, and averaged 24.6 million per clam.

11. The highest number of eggs released by any female clam at a single spawning was 24.3 million.

12. For clams, the correlation between number of eggs produced and volume of shell cavity was .38 (significant at the .05 level).

13. There was no significant difference in the average number of eggs released in a season whether the clams were spawned at three-, seven-, or 14-day intervals, nor was there any significant difference in the average number of spawnings per female.

14. The correlation between the number of times a female clam spawns and the number of eggs produced was not significantly different from zero.

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# AMERICAN SHAD, *ALOSA SAPIDISSIMA*, PARASITIZED BY *ARGULUS CANADENSIS* IN THE CONNECTICUT RIVER

Ectoparasitic branchiurans were collected on June 17, 1955 from shad in the Connecticut River which were being passed over the Holyoke dam by means of a fish elevator. The branchiurans were identified as *Argulus canadensis* Wilson from Wilson (1944, Proc. U. S. Nat. Mus. **94** (3177) : 568) by Dr. T. E. Bowman, Division of Marine Invertebrates, United States National Museum.

External parasites on shad from the Carolinas, the Woods Hole region, and the Connecticut River are mentioned by Yarrow (1874, Rept. U. S. Comm. Fish., 1872–1873, Part II: 456), Sumner, Osburn, and Cole (1913, Bull. U. S. Bur. Fish., 1911, **31**, Part II: 742), and Mansuetti and Kolb (1953, Chesapeake Biol. Lab., Pub. No. 97, Dec.: 19) respectively. The present report is the first one specifically listing *Alosa sapidissima* as a host for *Argulus canadensis*.

*Argulus canadensis* were first noted on some of the shad on June 11, nineteen days after the first fish in the spawning run had reached the Holyoke dam. They continued to be present on most of the 1668 shad passed between that date and June 28 when the run terminated.

On June 17 spawned out shad, called backrunners, began to enter the Holyoke canal system and congregate near the surface of the water in front of trash racks at industrial plants and power stations. Thirteen days later the first parasitized backrunner was captured. It is of interest that parasitized shad were not present among either the early upstream or downstream migrants.

When first observed on upstream migrants, the parasites were concentrated chiefly on the cheeks immediately ventral and posterior to the eyes. By June 20 they were common on the sides as well as the heads of the fish, particularly around lesions. The cause of these lesions was not determined, but similar ones were seen on unparasitized fish. Parasites were located on backrunners in positions similar to those on late upstream migrants.

Other fishes taken with shad in the fish elevator were not infected with *Argulus canadensis*, although this parasite has been reported from Lake of the Woods, Le Claire, Minn., on a species of whitefish, *Coregonus*, and on a rock sturgeon, *Acipenser fulvescens*, by Wilson (1916, Bull. U. S. Bur. Fish., 1914, **34**: 348). Wilson (1936, Canada Biol. Board Journ. **2**: 355) also reported it from Lake Ainslie and the Margaree River on Cape Breton Island, Nova Scotia, chiefly on brook trout although a few were found on stickle-backs, perch, and suckers. The United States National Museum has records of *Argulus canadensis* from *Coregonus sp.* Lake of the Woods, Canada and Minn.; *Coregonus clupeaformis* near Quebec City, Canada; *Acipenser fulvescens* Lake of the Woods, Canada; *Salvelinus fontinalis* Cape Breton Island, Nova Scotia, and Lake Kenogami, Quebec, Canada; *Gasterosteus wheatlandi* Cape Breton Island, Nova Scotia; and *Esox masquinongy* Trout Lake, Vilas Co., Wis.

Specimens of *Argulus canadensis* collected from *Alosa sapidissima* in the Connecticut River are in the United States National Museum with Catalogue Number 98464.—WILLIAM S. DAVIS, *U. S. Fishery Laboratory, Beaufort, North Carolina.*

# The Early Life History of the Lake Trout in Lake Superior

*By*

**PAUL H. ESCHMEYER**

MICHIGAN DEPARTMENT OF CONSERVATION
INSTITUTE FOR FISHERIES RESEARCH
ANN ARBOR, MICHIGAN

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# THE EARLY LIFE HISTORY OF THE LAKE TROUT IN LAKE SUPERIOR

*By*

*Paul H. Eschmeyer*[1]

## INTRODUCTION

The lake trout *(Salvelinus namaycush)* has been of outstanding importance throughout the history of commercial fishing in the upper Great Lakes. For many years it supported an annual production of about 15 million pounds and led all species in the value of the catch. It was important in the sport fishery of these waters as well, and still supports a substantial "deep-sea-trolling" fishery in Lake Superior.

Despite the lake trout's importance as a commercial and sport species, relatively little is known about its biology. Published information is especially scarce on its early life history in either the Great Lakes or in other waters. Young of the year rarely have been collected after leaving the spawning grounds, and collections of yearling fish, which often are too small to be taken in the types of collecting gear ordinarily used in the deep water where they live, also are unusual.

Research on the lake trout of the Great Lakes was stimulated by the decline or collapse of the lake trout fisheries of Lake Huron (Hile, 1949) and Lake Michigan (Hile, Eschmeyer, and Lunger, 1951a), and the serious threat to the fishery of Lake Superior (Hile, Eschmeyer, and Lunger, 1951b) which followed the invasion of the upper Great Lakes by the parasitic sea lamprey *(Petromyzon marinus)*. Data on various aspects of the biology of the species were presented by Van Oosten (1950), Eschmeyer, Daly, and Erkkila (1953), Eschmeyer (1955), Cable (1956), Eschmeyer (1956), and Van Oosten and Eschmeyer (1956).

To obtain further information, particularly on the early life history, the U. S. Fish and Wildlife Service research vessel *Cisco* operated in Lake Superior in August 1952 and May–October 1953. Some of the resulting collections of small lake trout are the basis for the present paper. Data are presented on geographic and bathymetric distribution, growth, sex ratio, food, and associates of lake trout during their first 3 years of life (age-groups O, I, and II).

## MATERIALS AND METHODS

Young-of-the-year and 1- and 2-year-old lake trout were collected by dragging an otter trawl along the bottom. Most were caught in nets 35 feet wide at the wings and 36 feet long. Mesh sizes of the cotton netting ranged from 1½ and 2½ inches (extension measure) at the forward end to ½ inch at the cod end. Ordinarily the trawl was towed for 10 minutes (usually parallel to the contour). Water depth was recorded by a fathometer and station locations were determined by radar.[2] The distance covered in a 10-minute haul depended partly on wind and currents, but probably averaged about ½ mile.

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[1] This work was completed while the author was a member of the staff of Great Lakes Fishery Investigations, Fish and Wildlife Service, U. S. Department of the Interior.

[2] A description of the *Cisco* and its navigational and research equipment was given by Moffett (1954).

Trawl collections of 2-year-old lake trout were supplemented by catches in gangs of experimental nylon gill nets of graded mesh. Most were caught in nets of 1-inch mesh, the smallest size fished. Young of the year and yearlings were not taken in this gear.

Nearly all lake trout were measured soon after capture, before preservation in formalin. Correction factors based on measurements of sizable samples of small lake trout before and after preservation were applied to the remainder. Thus, all measurements are for fish in the fresh condition. Lengths are total except where indicated otherwise. Mesh sizes of gill nets are extension measure.

For the purposes of this paper the terms young of the year, first summer, or O group designate fish in their first year of life. Yearling, I-group, or 1-year-old trout are in their second year, and II-group or 2-year-old fish are in their third year of life. Fish are moved to the next higher age group on January 1.

The following common and scientific names designate the species of fish to which reference is made in the text and tables:

Sea lamprey, *Petromyzon marinus*
Lake trout, *Salvelinus namaycush*
Shallowwater cisco, *Coregonus artedii*
Shortnose cisco, *Coregonus reighardi*
Shortjaw cisco, *Coregonus zenithicus*
Bloater, *Coregonus hoyi*
Kiyi, *Coregonus kiyi*
Lake whitefish, *Coregonus clupeaformis*
Round whitefish, *Coregonus cylindraceus*
Pygmy whitefish, *Coregonus coulteri*
American smelt, *Osmerus mordax*
White sucker, *Catostomus commersoni*
Longnose sucker, *Catostomus catostomus*
Mudminnow, *Umbra limi*
Trout-perch, *Percopsis omiscomaycus*
Brook stickleback, *Eucalia inconstans*
Ninespine stickleback, *Pungitius pungitius*
Burbot, *Lota lota*
Yellow perch, *Perca flavescens*
Johnny darter, *Etheostoma nigrum*
Fourhorn sculpin, *Myoxocephalus quadricornis*
Spoonhead sculpin, *Cottus ricei*
Slimy sculpin, *Cottus cognatus*

An additional species which may have been collected but which was not identified is the blackfin cisco, *Coregonus nigripinnis*.

## Local and Annual Differences in Abundance

*Variations in abundance of young of the year.*—First-summer lake trout were collected at eight stations in southern Lake Superior, from the Apostle Islands eastward to a point near Grand Marais, Michigan (Table 1, Fig. 1). Unquestionably they occurred in many other localities near Lake Superior's widespread lake trout spawning grounds. Collecting was restricted to areas with a suitable bottom for trawling where commercial fishing gear was not in use. No trawling was attempted along the entire northern and most of the eastern shore of the lake.

Most O-group lake trout were caught off Laughing Fish Point (Station 10) where 338 (80 percent) of the 420 collected in 1952–1953 were caught. Of the remainder, 68 (16 percent) were caught in Keweenaw Bay (Stations 5, 7, and 8). With the exception of 9 fish caught near the Apostle Islands (Station 4) on July 29, 1953, collections at other stations were limited to 1 to 3 individuals. At nine widely scattered stations a minimum of 20 minutes of trawling failed to collect young-of-the-year trout, even though the fishing included depths within the bathymetric range in which O-group trout were caught off Laughing Fish Point, and dates later than June 22–23, when the earliest collections were made in 1953.

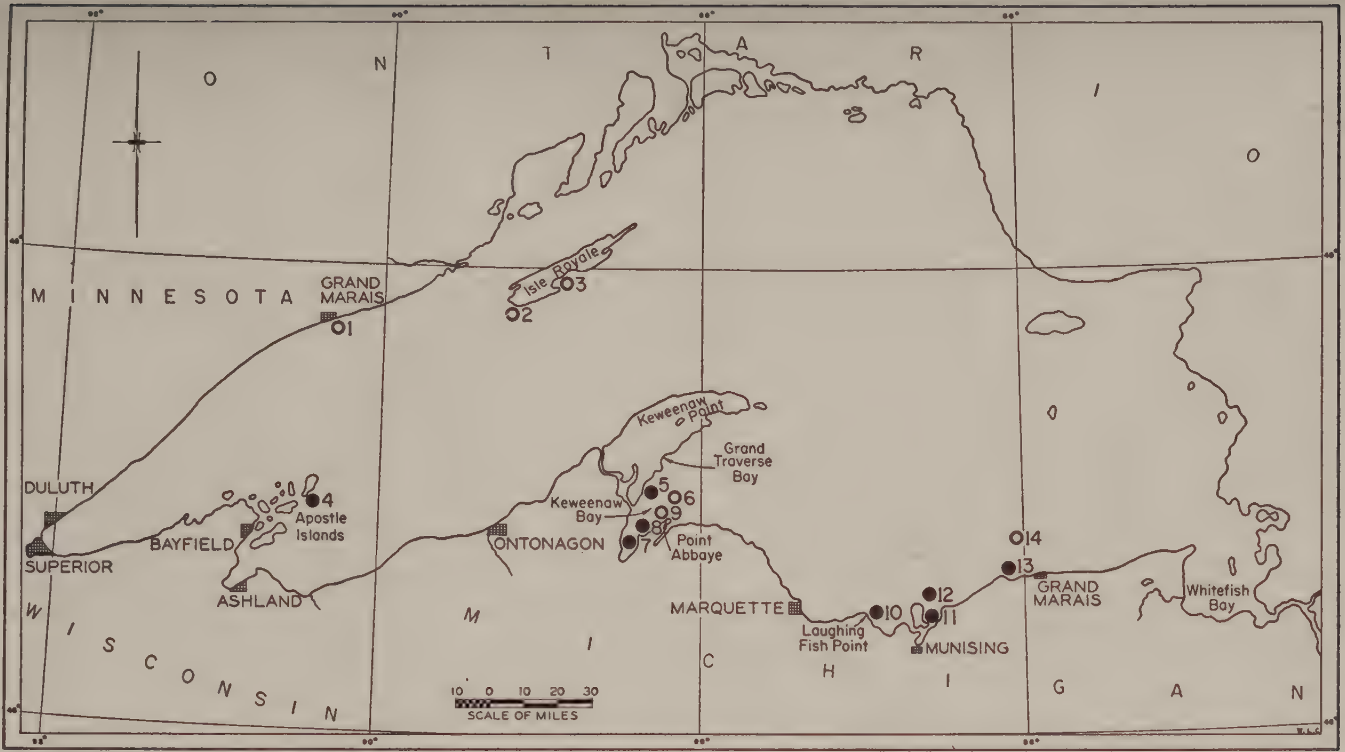
O N T A R I O
M I N N E S O T A
GRAND MARAIS
1
2
3
Isle Royale
DULUTH
SUPERIOR
BAYFIELD
4
Apostle Islands
ASHLAND
ONTONAGON
Keweenaw Point
Grand Traverse Bay
Keweenaw Bay
5
6
7
8
9
Point Abbaye
MARQUETTE
Laughing Fish Point
10
11
12
MUNISING
13
14
GRAND MARAIS
Whitefish Bay
W I S C O N S I N
M I C H I G A N
10 0 10 20 30
SCALE OF MILES

Figure 1.—Lake Superior, showing collecting stations for small lake trout and localities of special interest in this study. Solid circles indicates trawling stations where young of the year were collected. Open circles indicate additional collecting stations for 1- and/or 2-year-old lake trout.

Table 1.—Collection localities for O-group lake trout in Lake Superior, 1952—1953

| Locality* and date | Depth (fathoms) | Time trawled (minutes) | Number of fish |
|---|---|---|---|
| **Wisconsin** | | | |
| Apostle Islands (4) | | | |
| July 29, 1953 | 4–9 | 42 | 9 |
| **Michigan** | | | |
| Western Keweenaw Bay (5) | | | |
| Aug. 16, 1952 | 11–14 | 10 | 2 |
| Aug. 24–25, 1952 | 7–17 | 138 | 55 |
| July 23–24, 1953 | 11–14 | 36 | 1 |
| Aug. 3, 1953 | 10–13 | 18 | 1 |
| Sept. 5, 1953 | 10–17 | 29 | 1 |
| Southern Keweenaw Bay (7) | | | |
| Oct. 2–3, 1953 | 14–32 | 43 | 1 |
| Oct. 15, 1953 | 14–45 | 135 | 2 |
| Eastern Keweenaw Bay (8) | | | |
| Aug. 25, 1952 | 9–29 | 58 | 3 |
| July 11, 1953 | 10–18 | 65 | 1 |
| Sept. 10, 1953 | 6–18 | 60 | 1 |
| Laughing Fish Point (10) | | | |
| Aug. 27, 1952 | 13–29 | 93 | 30 |
| June 22–23, 1953 | 12–16 | 64 | 29 |
| July 13, 1953 | 18–23 | 40 | 10 |
| July 22, 1953 | 16–18 | 35 | 8 |
| Aug. 12, 1953 | 11–22 | 42 | 48 |
| Aug. 24, 1953 | 14–18 | 44 | 75 |
| Sept. 14, 1953 | 13–39 | 120 | 73 |
| Oct. 5, 1953 | 15–30 | 50 | 33 |
| Oct. 21–22, 1953 | 13–40 | 103 | 32 |
| North of Munising (11) | | | |
| Aug. 14, 1953 | 7–13 | 30 | 2 |
| Aug. 23, 1953 | 21–22 | 10 | 1 |
| North of Munising (12) | | | |
| Aug. 14, 1953 | 8–14 | 12 | 1 |
| West of Grand Marais (13) | | | |
| Aug. 15, 1953 | 9–11 | 40 | 1 |

* Station numbers in parentheses.

The numbers of young-of-the-year lake trout taken at the several stations are not directly indicative of the abundance in these areas because of variation in fishing time. Nevertheless, first-summer fish appeared to be markedly more plentiful off Laughing Fish Point in 1953 than in any other area. Most were taken here over a sandy bottom, outside an extensive rocky area which is well known to commercial fishermen as a lake trout spawning ground.

Young-of-the-year lake trout were decidedly more abundant in western Keweenaw Bay (Station 5) in 1952 than in 1953. In the former year, 148 minutes of trawling, August 16–25, yielded 57 lake trout, or an average of 3.9 per 10-minute haul; between July 8 and September 9, 1953, 105 minutes of fishing within the same depth interval (5–19 fathoms) yielded only 3 fish (0.3 per haul). No similar difference in rate of capture was observed off Laughing Fish Point (Station 10), the other principal collection locality. Here O-group lake trout were possibly more plentiful in 1953 than in 1952, although their numbers seemed to be at similar levels during the two years.

The infrequency of capture of young-of-the-year lake trout in large numbers in a single drag of the trawl suggests that they did not occur in large schools. Although as many as 60 were caught in one haul and 30 in another (both in August 1953), 10 or more were caught in only 10 hauls in the two seasons of collection. In contrast, a single 10-minute haul yielded as many as 3,511 shallowwater ciscoes, 2,332 ninespine sticklebacks, 972 trout-perch, 778 cottids, or 356 smelt.

*Local variations in abundance of I- and II-group lake trout.*—Most yearling lake trout were caught in southern Keweenaw Bay. Of 169 collected in 1952–1953, 153 (91 percent) were taken in this area (150 at Station 7, at the southern end of the Bay). Twelve were caught off Laughing Fish Point, 3 in Siskiwit Bay, Isle Royale (Station 3) and 1 in Munising Bay (Station 11). Still others were captured in the Apostle Islands (south and west of Station 4), but a large proportion of these were fin-clipped, hatchery-reared fish which had been released by the Wisconsin Conservation Department. Unmarked lake trout also had been stocked. To limit this study, in so far as possible, to native fish, records for collections in the Apostle Islands were not considered (except for the young of the year caught at Station 4).

Yearling lake trout, like the young of the year, were seldom taken in numbers in a single drag of the trawl, although as many as 15 were caught in each of four hauls in southern Keweenaw Bay, where they were more plentiful than in other waters fished. Many grounds which were undoubtedly used by lake trout of this size were too stony to be fished with trawls. The bottom in Keweenaw Bay was composed principally of clay, sand, and silt.

Most 2-year-old lake trout taken in trawls were caught in Keweenaw Bay, along with yearlings. Of 104 collected with trawls, 86 were caught in this area. Eleven of the remainder were caught in Munising Bay (Station 11) and the others at scattered localities. Trawl collections were supplemented by catches in 1-inch-mesh gill nets (a few were captured in nets of larger mesh), principally in Keweenaw Bay (110 fish) and off Isle Royale (100).

## BATHYMETRIC DISTRIBUTION

*Bathymetric distribution of young of the year.*—Most of the young-of-the-year lake trout caught off Laughing Fish Point, the principal place of collection, were taken at depths less than 20 fathoms from late June until late August (Table 2). On dates when collections were made at both the 10–14-fathom and 15–19-fathom intervals, the rate of capture was higher in the shallower of these depth strata. This observation was supported by collections at Keweenaw Bay (Station 5) on August 24–25, 1952, where young lake trout were more plentiful at the 10-14-fathom interval than at 5–9 or 15–19 fathoms. Although waters shallower than 10 fathoms were seldom fished, trawling in the Apostle Island region (Station 4) on July 29, 1953, was more productive at 5–9 fathoms (3.6 fish per haul) than at shallower depths (0.5 per haul). Deeper waters were not trawled at this station because of the rocky bottoms.

One of the season's most severe storms occurred in Lake Superior on September 12 and 13, 1953, with northwest winds heavy enough to ground an ore carrier near Laughing Fish Point and to cause other damage to shipping. The resulting heavy seas may have caused the marked change in bathymetric distribution of small lake trout taken on September 14. On this date O-group lake trout were caught at all depths fished between 13 and 39 fathoms, and appeared to be more plentiful at 30–39 fathoms than at other depths. Although waters deeper than 25–29 fathoms were not fished on October 5, the records suggest that young trout may have become redistributed at the 20–24-fathom interval by that date, and at 25–29 fathoms by October 21–22.

**Table 2.—Numbers of O-group lake trout caught per 10-minute drag of an otter trawl off Laughing Fish Point (Station 10), Lake Superior, 1952–1954**

[Number of minutes of trawling in parentheses. All dates are 1953 except where otherwise indicated]

| Depth* (fathoms) | June 22–23 | July 13 | July 22 | Aug. 12 | Aug. 24 | Aug. 27, 1952 | Sept. 14 | Oct. 5 | Oct. 21–22 | Jan. 7, 11, 1954 |
|---|---|---|---|---|---|---|---|---|---|---|
| 10–14 | 5.0<br>(28) | .. | .. | 27.5<br>(12) | .. | 5.8<br>(19) | 1.0<br>(10) | .. | 0.0<br>(10) | .. |
| 15–19 | 4.2<br>(36) | 3.3<br>(30) | 2.3<br>(35) | 7.5<br>(20) | 17.0<br>(44) | 4.4<br>(39) | 4.8<br>(40) | 0.0<br>(10) | 1.0<br>(10) | .. |
| 20–24 | .. | 0.0<br>(10) | .. | 0.0<br>(10) | .. | 0.6<br>(31) | 6.8<br>(40) | 15.0<br>(20) | 3.6<br>(33) | .. |
| 25–29 | .. | .. | .. | .. | .. | 0.0<br>(4) | 3.0<br>(10) | 1.5<br>(20) | 7.2<br>(25) | 0.0<br>(20) |
| 30–34 | .. | .. | .. | .. | .. | .. | 8.0<br>(10) | .. | 0.7<br>(15) | .. |
| 35–39 | .. | .. | .. | .. | .. | .. | 15.0<br>(10) | .. | 0.0<br>(10) | 0.0<br>(10) |
| 40–44 | .. | .. | .. | .. | .. | .. | .. | .. | .. | 0.0<br>(10) |
| 45–49 | .. | .. | .. | .. | .. | .. | .. | .. | .. | 0.5<br>(20) |
| 50–54 | .. | .. | .. | .. | .. | .. | .. | .. | .. | 0.0<br>(10) |
| Total number | 29 | 10 | 8 | 48 | 75 | 30 | 73 | 33 | 32 | 1 |

* Placement of individual hauls based on the mean of the shallowest and deepest water fished. Most tows paralleled isobaths as nearly as possible.

On January 7 and 11, 1954, yearling[3] lake trout were extremely scarce near Laughing Fish Point. Seventy minutes of trawling at depths between 26 and 54 fathoms yielded only one fish, at the 45–49-fathom interval.

Observations on the seasonal depth distribution of young lake trout near Laughing Fish Point were corroborated closely by more limited collections in other areas. In the combined collections at all stations in 1952 and 1953, only five young lake trout were caught at a depth of 20 fathoms or more before September 14; only one was taken at less than 20 fathoms after that date. Lake trout in southern Lake Superior apparently spent most of their first summer in relatively shallow water, not far from the spawning grounds on which they were hatched, until equinoctial storms forced them to seek greater depths.

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[3] Young of the year commonly would be regarded as yearlings after December 31.

In contrast to the open-water habitat of young-of-the-year lake trout in Lake Superior, Kendall and Goldsborough (1908) collected 7 young, 1.9 to 2.4 inches long (possibly recently stocked), in small tributaries of First Lake, Connecticut. Neave and Bajkov (1929) took 10 young, 1.3 to 1.8 inches long, in a small inlet of Pyramid Lake, Alberta. A single fingerling, 2.3 inches long, was caught in the Ocqueoc River, Presque Isle County, Michigan, a tributary of Lake Huron, by personnel of the U. S. Fish and Wildlife Service, on August 20, 1951. In Great Bear Lake, Northwest Territory, Canada, Miller and Kennedy (1948) collected 14 young (average fork length, 1.1 inches) and observed many others along the rocky shores of bays, in water only a few inches deep, where they were associated with 1-, 2-, and 3-year-old lake trout.

Two young-of-the-year lake trout have been collected in New York waters in a habitat comparable to that in which they were caught in Lake Superior. Greeley (1929) took a 2.3-inch lake trout September 19, 1926, from 60 feet of water in Hemlock Lake; and Royce (1951) caught a single advanced fry in about 130 feet of water on April 2, 1940, in Seneca Lake.

*Bathymetric distribution of I- and II-group lake trout.*—The bathymetric distribution of yearling and of 2-year-old lake trout collected by trawling in southern Keweenaw Bay (Station 7), where most were caught, was so similar that data for the two age groups were combined (Table 3). The average depth of capture for 150 yearlings was 30 fathoms and for 86 II-group lake trout it was 32 fathoms. The bathymetric distribution of fish of both age groups ranged from 15 to 49 fathoms during the season and both were most plentiful at 20–34 fathoms, except on May 10–11, when the only fish caught in trawls were taken at 40–49 fathoms. Undoubtedly additional trawling at shallower depths in May would have taken small trout, because 2-year-old fish were caught throughout a depth range of 29–46 fathoms in gill nets lifted in northern Keweenaw Bay (Station 6) on May 7–9. The scarcity of I- and II-group lake trout in Keweenaw Bay on August 2, when only 11 were caught in 2 hours of trawling, is unexplained.

No appreciable shift in bathymetric distribution of small lake trout occurred between July 11 and October 15; all collections showed the greatest concentrations of fish within the 20–34-fathom interval. This observation was supported strongly by collections in other areas. Of 35 I- and II-group lake trout caught in trawls at other stations in 1953, 29 (83 percent) were taken at 20–34 fathoms. Gill-net catches both in Keweenaw Bay and near Isle Royale, as well as at other stations in Lake Superior, showed good agreement with trawling results in demonstrating the relatively greater abundance of II-group lake trout in this stratum than at other depths.

In other Great Lakes waters small lake trout have been collected at depths comparable to those at which they were taken in Lake Superior. Dymond, Hart, and Pritchard (1929) recorded the capture in Lake Ontario of 11 lake trout, 5 to 6 inches long, at a depth of 25 fathoms, and

**Table 3.—Numbers of I- and II-group lake trout caught per 10-minute drag of an otter trawl in southern Keweenaw Bay (Station 7), Lake Superior, 1953**

[Number of minutes of trawling in parentheses]

| Depth* (fathoms) | May 10–11 | July 11 | Aug. 2 | Sept. 11 | Oct. 2–3 | Oct. 15 | Average |
|---|---|---|---|---|---|---|---|
| 10–14 | .. | .. | 0.0<br>(20) | 0.0<br>(10) | .. | 0.0<br>(10) | 0.0<br>(40) |
| 15–19 | .. | .. | 0.0<br>(20) | 0.0<br>(10) | 2.0<br>(10) | .. | 0.5<br>(40) |
| 20–24 | .. | .. | .. | 2.0<br>(10) | 12.0<br>(20) | 1.5<br>(20) | 5.8<br>(50) |
| 25–29 | .. | 0.0<br>(10) | 1.3<br>(30) | 9.7<br>(35) | .. | 5.6<br>(75) | 5.3<br>(150) |
| 30–34 | .. | 24.0<br>(20) | 1.8<br>(40) | 6.9<br>(39) | 8.5<br>(13) | .. | 8.3<br>(112) |
| 35–39 | 0.0<br>(6) | .. | 0.0<br>(10) | .. | .. | 1.0<br>(10) | 0.4<br>(26) |
| 40–44 | 2.2<br>(77) | .. | .. | 1.0<br>(10) | .. | 0.5<br>(20) | 1.8<br>(107) |
| 45–49 | 1.6<br>(75) | .. | .. | .. | .. | .. | 1.6<br>(75) |
| Total number | 29 | 48 | 11 | 64 | 37 | 47 | 236 |

* Placement of individual hauls based on the mean of the shallowest and deepest water fished. Most tows paralleled isobaths as nearly as possible.

one 8½-inch fish at 17 fathoms. Van Oosten and Eschmeyer (1956) reported the collection of a 6.9-inch fish at a depth of 34 fathoms in Lake Michigan. They showed that juvenile lake trout (mostly 12 to 15 inches long) were concentrated at 40–49 fathoms in southern Lake Michigan and at 30–39 fathoms in the northern region of the lake.

In other waters, Royce (1951) caught 9 lake trout less than 9 inches long in water 40 to 70 feet deep in Keuka Lake, New York. Martin (1952) found lake trout distributed throughout an extensive range of depths (including shallow water) in Lake Louisa and Redrock Lake, Ontario. Seasonal changes in distribution occurred, apparently in response to thermal stratification. In general, small lake trout were in deeper waters than the larger fish. In Great Bear Lake, Northwest Territory, Canada, Miller and Kennedy (1948) reported that lake trout spent the first four summers of life in shallow water, along a rocky shoreline.

*Relation of water temperatures to bathymetric distribution.*—Water temperatures near Laughing Fish Point at the depths and on the dates when young-of-the-year lake trout were collected in 1953 ranged from 42° to 63°F. (Table 4). Temperatures at depths of 10 and 15 fathoms were slightly lower on July 22 than on the previous dates of collection (June 22–23 and July 13), but the data suggest a progressive warming of the water at depths greater than 5 fathoms from July 22 to September 14.

Although many temperature changes may have occurred during the 3-week period, August 24–September 14, it is likely that the nearly homothermous conditions in the upper 20 fathoms on the latter date resulted from the severe storm of September 12–13. Besides the extensive mixing of the water which the heavy seas must have caused, warm surface waters were probably moved into the area by the northwest winds, and cold waters moved out of the deeper strata by the resulting submarine currents. These hydrographic changes may explain the unusual bathymetric distribution of lake trout on September 14 (Table 2). The change from August 24 to September 14 may have been caused more by the movement of water masses which the fish occupied than by migration or movement of the fish themselves. Under comparable circumstances, Hile and Deason (1947) associated marked deviations from the normal bathymetric distribution of the kiyi in Lake Michigan with unusual hydrographic conditions, which could have caused the disturbance of considerable masses of water.

**Table 4.—Water temperatures (°F.) at different depths and localities in Lake Superior, 1953**

[Data from bathythermograph records. When two or more casts were made at the same station on the same date, average temperatures are shown]

| Locality* and date | Depth (fathoms) | | | | | | |
|---|---|---|---|---|---|---|---|
| | S** | 5 | 10 | 15 | 20 | 25 | 30 |
| **Laughing Fish Point** (10) | | | | | | | |
| May 5 | 38 | 38 | 38 | 38 | .. | .. | .. |
| June 12 | 44 | 43 | 41 | 41 | 41 | .. | .. |
| June 22–23 | 48 | 48 | 47 | 46 | .. | .. | .. |
| July 13 | 56 | 56 | 53 | 49 | .. | .. | .. |
| July 22 | 62 | 50 | 46 | 42 | .. | .. | .. |
| Aug. 12–13 | 62 | 56 | 47 | 44 | .. | .. | .. |
| Aug. 24 | 67 | 65 | 58 | 45 | .. | .. | .. |
| Sept. 14 | 63 | 63 | 63 | 63 | 62 | 53 | 46 |
| Oct. 5 | 56 | 56 | 56 | 56 | .. | .. | .. |
| Oct. 21 | 55 | 54 | 53 | 50 | 48 | 46 | 44 |
| **Keweenaw Bay** (7) | | | | | | | |
| May 11 | 39 | 39 | 39 | 39 | 39 | 39 | 39 |
| July 11 | 57 | 47 | 45 | 44 | 43 | 42 | 42 |
| Aug. 2 | 60 | 60 | 59 | 59 | 53 | 50 | 47 |
| Sept. 11 | 65 | 64 | 63 | 58 | 53 | 50 | 46 |
| Oct. 2–3 | 56 | 53 | 53 | 51 | 50 | 49 | 49 |
| Oct. 15 | 53 | 53 | 52 | 47 | 45 | 43 | 42 |
| **Isle Royale** (2, 3) | | | | | | | |
| July 25 | 53 | 43 | 40 | 39 | 39 | 39 | .. |
| Sept. 7–8 | 48 | 46 | 40 | 40 | 39 | 39 | 39 |
| Sept. 27–Oct. 1 | 49 | 49 | 48 | 45 | 43 | 42 | 41 |

* Station numbers in parentheses.
** Surface.

The homothermous conditions at Laughing Fish Point on September 14 persisted on October 5, although water temperatures were lower on the latter date. A further drop in temperature occurred by October 21. Although the zone of concentration of young trout was deeper in October than earlier in the season (Table 2) the temperature of the water which they occupied was roughly similar to that in which they were found in August.

Water temperatures ranged from 39° to 53°F. in the 20–34-fathom depths at which I- and II-group lake trout were most plentiful in Keweenaw Bay (Table 4). The higher temperature was recorded on both August 2 and September 11, at 20 fathoms; progressively cooler temperatures occurred in deeper water and on collection dates before and after this period. At Stations 2 and 3, off Isle Royale, water temperatures ranged from 39° to 43° F. at depths of 20–30 fathoms between July 25 and October 1. Possibly the water temperatures in the depths at which most small lake trout were caught did not exceed 43°F. during the 1953 season in this region of Lake Superior.

## GROWTH

*Growth of the young of the year.*—First-summer lake trout in the 1953 collections from Laughing Fish Point, where most were caught, increased in average length from 1.2 inches (range, 1.1–1.3) on June 22–23 to 3.1 inches (range, 2.8–3.7) on October 21–22 (Table 5). The average weight on the latter date was about 0.1 ounce. The mean growth during this 4-month period was about 2.8 millimeters per week, but the rate of increase was not uniform during the season; about 65 percent of the increase occurred in the 54-day period, July 22–September 14. The average weekly growth increased from less than 1 millimeter, June 22–23 to July 22, to an average of 4 millimeters, July 22–September 14, and then decreased to 2.4 millimeters between September 14 and October 22.[4] A single lake trout caught January 11, 1954, was 4.1 inches long.

**Table 5.—Growth of O-group lake trout, Laughing Fish Point (Station 10), Lake Superior, 1953–1954**

| Date of collection | Number of fish | Total length (millimeters) | | Average total length (inches) | Average weight (grams) | Weekly increment in total length (millimeters) |
|---|---|---|---|---|---|---|
| | | Average | Range | | | |
| 1953 | | | | | | |
| June 22–23 | 29 | 31 | 28–33 | 1.2 | 0.1 | ... |
| July 13 | 10 | 34 | 31–36 | 1.3 | 0.2 | 1.0 |
| July 22 | 8 | 35 | 33–37 | 1.4 | 0.3 | 0.8 |
| Aug. 12 | 48 | 46 | 37–54 | 1.8 | 0.6 | 3.7 |
| Aug. 24 | 75 | 53 | 44–67 | 2.1 | 0.9 | 4.1 |
| Sept. 14 | 73 | 66 | 55–81 | 2.6 | 1.6 | 4.3 |
| Oct. 5 | 33 | 72 | 53–88 | 2.8 | 2.6 | 2.0 |
| Oct. 21–22 | 32 | 79 | 71–93 | 3.1 | 3.2 | 3.0 |
| 1954 | | | | | | |
| Jan. 11 | 1 | 104 | .. | 4.1 | 6.5 | 2.2 |

The July 22–September 14 period of fastest growth of O-group lake trout was one of increasing water temperatures (Table 4) at the depths at which the fish were collected (Table 2), although higher temperatures (perhaps of short duration) were recorded on July 13 than on the two subsequent collection dates. The decline in rate of growth after September 14 took place as water temperatures were decreasing. This decrease was accentuated somewhat by the movement of young trout to progressively deeper (and cooler) water in October.

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[4] Although July collections off Laughing Fish Point may have been too small to be fully representative, slow early growth of lake trout was verified by measurements of 7 collections from the Marquette, Michigan, hatchery of the Michigan Department of Conservation, in 1954. Samples of 30 to 77 young showed an average increase from 17 millimeters soon after hatching (February 9) to 30 millimeters on May 12 (1 millimeter per week). Two lake trout fry collected on April 7 and 15, 1953, from spawning grounds near Marquette, Michigan (about 20 miles west of Laughing Fish Point), were each 23 millimeters long. Comparison with the 31-millimeter average length of young trout collected off Laughing Fish Point on June 22–23 suggests an average increment of about 1 millimeter per week.

Seasonal changes in the growth rate of young-of-the-year lake trout off Laughing Fish Point are clearly apparent in the graphical presentation of the data (Fig. 2). Symbols showing average length of fish in collections from other stations (Table 1), although based on only one to nine specimens, suggest, by the consistency of their occurrence above the curve for fish from Laughing Fish Point, that first-year growth in 1953 was somewhat slower in this region than in other localities in Lake Superior. Such a regional difference in growth rate was clearly demonstrated by two collections of first-summer trout in August 1952 (designated by open circles in Fig. 2). The mean length of 55 trout collected in western Keweenaw Bay (Station 5) was 2.4 inches (range, 2.0–2.7); for 30 caught off Laughing Fish Point on August 27 it was 2.0 inches (range, 1.6–2.3). Although the extremes of the ranges in length overlapped, the difference in size of fish at the two stations was clearly apparent at the time of collection, and was statistically significant ($t=11.52$; $p=<0.005$).

*Growth of I- and II-group lake trout.*—The average length of yearling lake trout in Keweenaw Bay (Station 7) in 1953 was 3.9 inches (range, 3.5–4.5) on May 11–12 and 5.6 inches (range, 4.6–6.3) on October 15 (Table 6). Average weights on these dates were 0.2 and 0.7 ounce. The lengths of fish in six collections during the season suggested a relatively uniform rate of growth of about 0.08 inch (1.9 millimeters) per week during this 5-month period (Fig. 3). The absence of any appreciable seasonal change in growth rate of yearlings (as occurred in the O and II group) is unexplained.

Lake trout in each of three small collections of yearlings from Laughing Fish Point (Station 10) averaged slightly longer than fish of Keweenaw Bay, although the length ranges were similar. Three yearlings caught in Siskiwit Bay, Isle Royale (Station 3) were smaller than fish of this age taken on similar dates in other areas.

Lake trout of age-group II were collected in both trawls and gill nets. In the two largest collections of fish of this age, 108 of 194 fish in Keweenaw Bay and 100 of 102 from Isle Royale were taken in gill nets (93 percent in nets of 1-inch mesh). Twice, in Keweenaw Bay, collections of 2-year-old lake trout were made in both trawls and gill nets on similar dates (Table 6). In May the fish collected by trawling were slightly larger than those taken in gill nets; in early September, the reverse was true. The length frequencies of fish captured by the two gears were similar on both dates (Table 7) and both trawls and gill nets took fish of a wide range in size. Catches from both gears accordingly were combined to study the rate of growth of II-group lake trout.

The average length of 27 II-group lake trout caught May 7–12 in Keweenaw Bay was 6.7 inches (range, 6.2–7.6); the mean length of 30 taken October 2–16 was 8.0 inches (range, 6.7–9.2). Average weights on these dates were 1.4 and 1.9 ounces. The increase in mean length during this period was about 0.06 inch per week. The rate was somewhat slower than average from early May to July 11 and faster after that date.

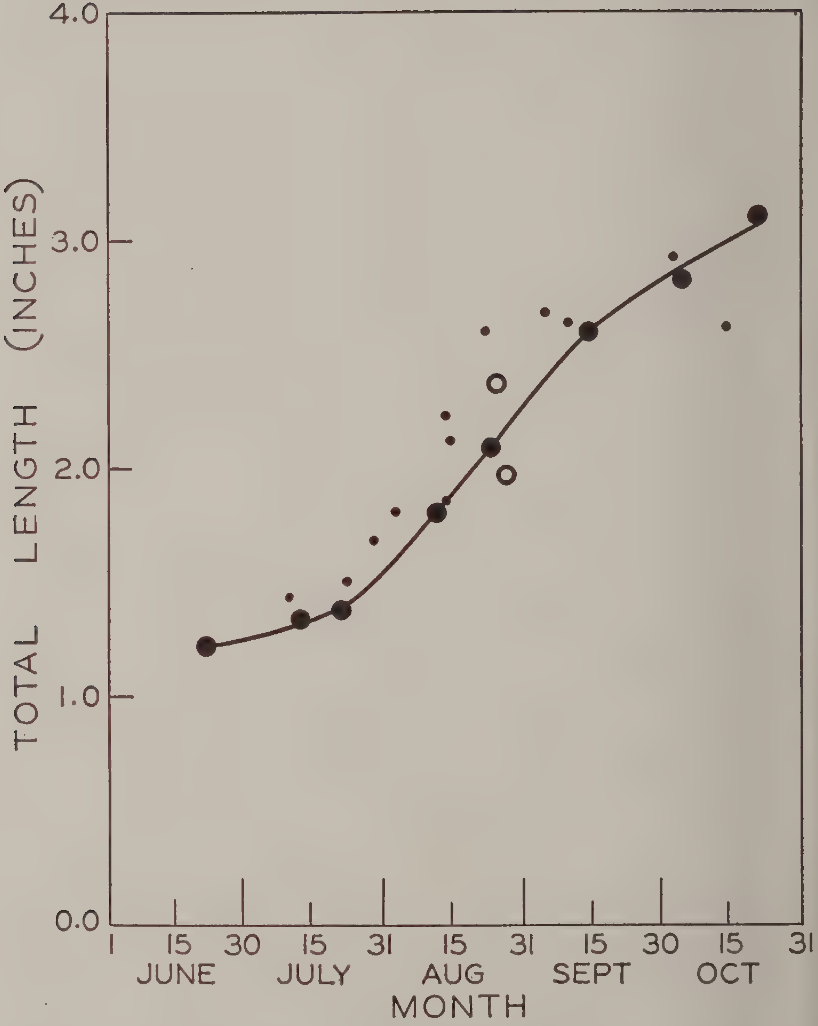

Figure 2.—Growth of young-of-the-year lake trout in Lake Superior, 1952–1953. The solid circles (on which the curve is based) are for collections off Laughing Fish Point (Station 10) in 1953. The open circles are for collections in 1952 from Keweenaw Bay (upper circle) and Laughing Fish Point (lower circle). The smaller dots represent collections of one to nine fish at various localities (see Table 1).

Table 6.—Collection localities, lengths, and weights of I- and II-group lake trout caught in trawls and gill nets in Lake Superior, 1952–1953

[All collection dates were in 1953 except where otherwise indicated. Seven yearlings caught singly on the following dates are not included in the table (station numbers in parentheses): May 10 (6), June 17 (11), June 23 and October 21 (10), July 8 (5), August 15, 1952 (10), and August 25, 1952 (8). Of the 2-year-olds, 3 caught in trawls and 3 taken in gill nets, at scattered localities and dates, are not included]

| Age, locality, and date | Station (Fig. 1) | Number of fish | Total length (inches) | | Average weight (ounces) |
|---|---|---|---|---|---|
| | | | Average | Range | |
| **Age-group I** | | | | | |
| Keweenaw Bay | | | | | |
| May 11–12 | 7 | 19 | 3.9 | 3.5–4.5 | 0.2 |
| July 11 | 7 | 32 | 4.6 | 4.2–5.4 | 0.4 |
| Aug. 2 | 7 | 5 | 4.7 | 4.4–5.3 | 0.4 |
| Sept. 11 | 7 | 37 | 5.2 | 4.4–6.0 | 0.5 |
| Oct. 2–3 | 7 | 27 | 5.5 | 4.8–5.9 | 0.7 |
| Oct. 15 | 7 | 30 | 5.6 | 4.6–6.3 | 0.7 |
| Laughing Fish Point | | | | | |
| Aug. 12 | 10 | 3 | 5.0 | 4.4–5.5 | 0.6 |
| Sept. 14 | 10 | 4 | 5.5 | 5.4–5.6 | 0.6 |
| Oct. 5 | 10 | 2 | 5.6 | 5.8–6.1 | 0.8 |
| Isle Royale | | | | | |
| Sept. 7 | 3 | 3 | 4.6 | 4.3–5.0 | 0.5 |
| **Age-group II** | | | | | |
| Keweenaw Bay | | | | | |
| Aug. 26, 1952 | 8 | *47 | 7.1 | 6.2–8.2 | 1.3 |
| May 7–9 | 6 | *17 | 6.6 | 6.2–7.3 | 1.4 |
| May 11–12 | 7 | 10 | 6.9 | 6.3–7.6 | 1.3 |
| July 11 | 7 | 16 | 6.9 | 6.1–7.8 | 1.3 |
| Aug. 2 | 7 | 6 | 7.1 | 6.2–7.7 | 1.6 |
| Sept. 3 | 9 | *39 | 7.6 | 6.8–8.3 | 1.6 |
| Sept. 10 | 9 | * 2 | 8.3 | 8.2–8.4 | 2.0 |
| Sept. 11 | 7 | 27 | 7.4 | 5.6–8.2 | 1.5 |
| Oct. 2 | 7 | 10 | 8.1 | 7.2–8.9 | 2.1 |
| Oct. 15 | 7 | 17 | 7.9 | 7.2–9.2 | 1.9 |
| Oct. 16 | 7 | * 3 | 7.5 | 6.7–8.5 | 1.7 |
| Laughing Fish Point | | | | | |
| Sept. 14 | 10 | 2 | 7.0 | 6.8–7.1 | 1.4 |
| Munising | | | | | |
| Aug. 15, 1952 | 12 | *16 | 7.0 | 6.4–7.5 | 1.3 |
| June 17–21 | 11 | 8 | 6.2 | 5.9–6.7 | 1.0 |
| June 14–22 | 11, 12 | * 8 | 6.6 | 6.3–7.1 | 1.1 |
| Aug. 14 | 11 | * 9 | 6.7 | 6.5–6.9 | 1.2 |
| Aug. 14 | 11 | 3 | 5.9 | 5.7–6.1 | 0.7 |
| Grand Marais, Michigan | | | | | |
| Aug. 14, 1952 | 14 | * 4 | 7.4 | 7.1–8.0 | 1.6 |
| June 15 | 14 | * 4 | 6.7 | 6.4–7.1 | 1.1 |
| Grand Marais, Minnesota | | | | | |
| July 27 | 1 | * 5 | 6.7 | 6.3–7.4 | 1.2 |
| Isle Royale | | | | | |
| July 26 | 2 | *14 | 6.3 | 5.8–6.9 | 1.0 |
| Sept. 7 | 3 | 2 | 6.8 | 6.7–6.8 | 1.1 |
| Sept. 8 | 2 | *29 | 6.6 | 6.0–7.7 | 1.1 |
| Sept. 29–Oct. 1 | 2,3 | *57 | 6.9 | 5.9–8.5 | 1.5 |

* Gill-net collections. All other fish were caught in trawls.

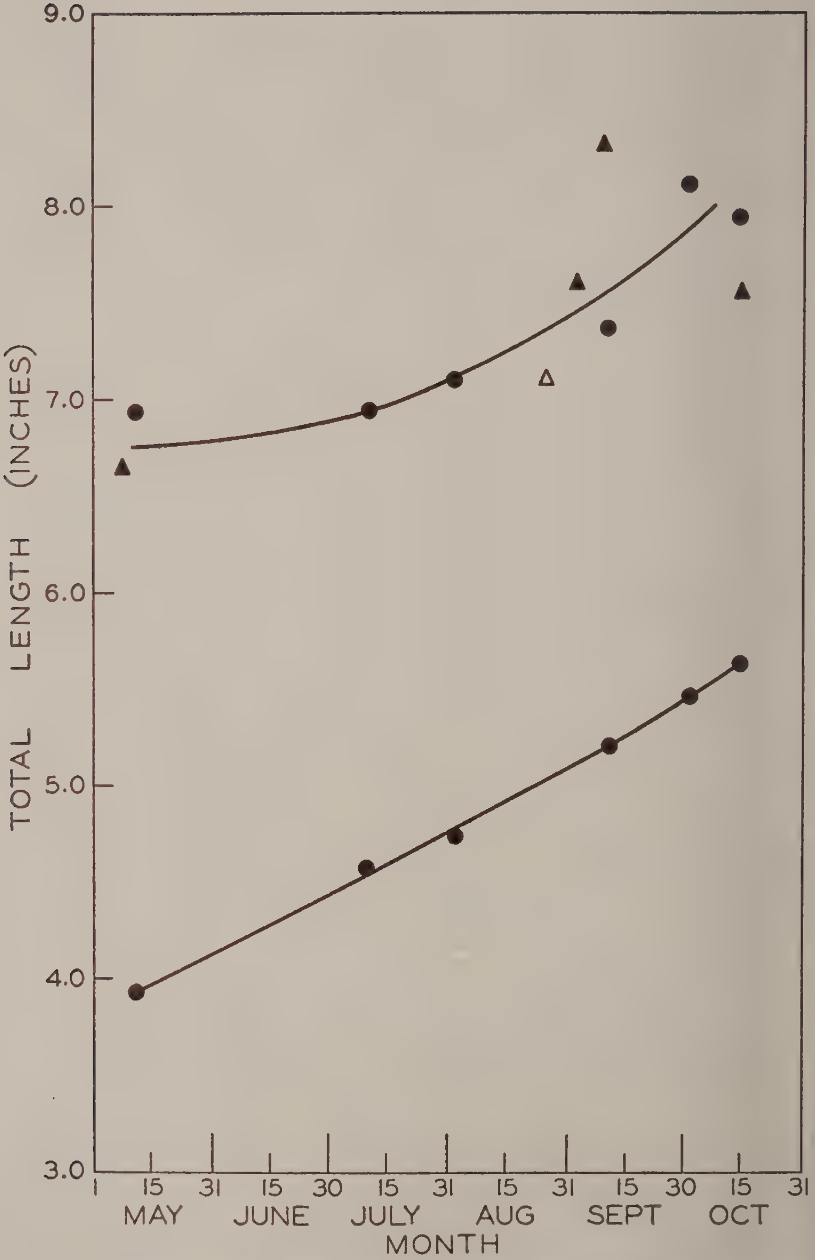

Figure 3.—Growth of yearling (lower curve) and 2-year-old (upper curve) lake trout in Lake Superior, 1952–1953. The solid circles and solid triangles (on which the curve is based) are for collections from trawls and gill nets, respectively, in Keweenaw Bay in 1953. The open triangle is for a gill-net collection in 1952.

Table 7.—Length-frequency distribution of 2-year-old lake trout caught in trawls and gill nets in Lake Superior, 1953

| Total length (inches) | Keweenaw Bay (Station 7) | | | | Isle Royale (Stations 2 and 3) | |
|---|---|---|---|---|---|---|
| | Trawl | Gill net | Trawl | Gill net | Gill net | |
| | May 11–12 | May 7–9 | Sept. 11 | Sept. 3 | Sept. 8 | Sept. 29–Oct. 1 |
| 5.6–5.7 | .. | .. | 1 | .. | .. | .. |
| 5.8–5.9 | .. | .. | .. | .. | .. | 1 |
| 6.0–6.1 | .. | .. | .. | .. | 1 | 2 |
| 6.2–6.3 | 2 | 4 | 1 | .. | 6 | 9 |
| 6.4–6.5 | 1 | 4 | 1 | .. | 8 | 7 |
| 6.6–6.7 | 1 | 2 | .. | .. | 4 | 11 |
| 6.8–6.9 | 2 | 4 | 1 | 1 | 6 | 7 |
| 7.0–7.1 | 1 | 2 | 3 | 1 | 2 | 3 |
| 7.2–7.3 | .. | .. | 6 | 8 | .. | 6 |
| 7.4–7.5 | 1 | 1 | 3 | 11 | 1 | 3 |
| 7.6–7.7 | 2 | .. | 3 | 6 | 1 | 1 |
| 7.8–7.9 | .. | .. | 3 | 6 | .. | 2 |
| 8.0–8.1 | .. | .. | 3 | 1 | .. | 2 |
| 8.2–8.3 | .. | .. | 2 | 5 | .. | 1 |
| 8.4–8.5 | .. | .. | .. | .. | .. | 2 |
| Number of fish | 10 | 17 | 27 | 39 | 29 | 57 |
| Average length | 6.9 | 6.6 | 7.4 | 7.6 | 6.6 | 6.9 |

Two-year-old lake trout collected in 1953 in the vicinity of Munising, Michigan, and Isle Royale, as well as from other scattered localities, were of smaller average size than in Keweenaw Bay. Among fish in the larger collections, those from Isle Royale were consistently the smallest. The average length of fish in both September gill-net collections off Isle Royale was markedly shorter than in a collection on September 3 in Keweenaw Bay taken with identical gear (Table 7).

The growth curve suggested by the three principal collections from Isle Royale, between July 26 and October 1, was roughly parallel to the corresponding portion of the curve for fish from Keweenaw Bay, although a slight divergence (toward slower growth in the Isle Royale area) was apparent.

*Progress of the season's growth.*—The 1953 season's growth of I- and II-group lake trout in Keweenaw Bay apparently began sometime between May 11 and July 11. New growth had not begun in May, but in July the scales of 41 percent of the I group and 28 percent of the II group showed some evidence of new growth (not always a complete annulus). The beginning of new-season's growth was observed in scales of each of 5 yearlings and 3 of 6 II-group fish from Keweenaw Bay on August 2 and in 7 of 14 II-group trout from Isle Royale on July 26; the scales of nearly all small lake trout collected on later dates showed some growth during the current season.

The period of the year during which collections were made in 1953 unquestionably represented only a portion of the growing season for each of the first three age groups of lake trout. This belief is supported by the wide gaps between the average lengths and weights of fish collected in

October and those of the next oldest age group collected in May (before the new season's growth had begun). Although some annual fluctuations in growth rate might occur, it would hardly be expected to reach the magnitude suggested by these discrepancies.[5] Thus, young-of-the-year lake trout in Keweenaw Bay must have attained an average length of 3.9 inches in the 1952 growing season; the yearlings, 6.7 inches; and 2-year-olds, 8.6 inches (the last value was the average length of 18 III-group fish collected in gill nets and trawls in Keweenaw Bay, May 7–12, 1953). For fish sampled in 1953 to have attained similar lengths by May 1954, substantial growth would have to be made subsequent to the last collections in October. This amount was 0.8 inch for the O group (20 percent of the total growth of fish of this age in Keweenaw Bay in 1952), 1.1 inches (39 percent of the presumed 1952 growth) for the I group, and 0.6 inch (32 percent) for the II group. If growth rate in 1953 had continued at the rate suggested by September and October collections (Tables 5 and 6, Fig. 3), young lake trout would have attained their "expected" average lengths in late December, yearlings in mid-January, and II-group fish by late November. Since this rate was probably not maintained (the structure of scales from most small lake trout suggests a reduction in growth rate late in each growing season), the data strongly suggest that small lake trout may continue to grow to or beyond the end of the calendar year.

Cable (1956) reported that growth of lake trout in Lake Michigan may continue past the end of the calendar year during at least some seasons, and Fry (1953) showed that growth of lake trout in South Bay, Lake Huron, continued at least until mid-October. In contrast, Kennedy (1954) wrote that growth in Great Slave Lake was confined to the period from late May to mid-September.

*Length as an indicator of age.*—Lake trout from each of the first three age groups in Lake Superior showed rather uniform growth, with little overlap in the length range of fish of different ages collected at comparable dates and localities. Lengths of O-group fish did not overlap those of the I group; for fish from Keweenaw Bay the length ranges of the I and II group overlapped only in one collection (September 11), and only one fish was involved. By mid-October the extremes of the length ranges of fish of the I and II groups had converged somewhat, however, suggesting that length alone might not be a safe criterion for separating fish of older age groups.

*Growth of small lake trout in other waters.*—The available data on the rate of growth of small lake trout in Keweenaw Bay, Lake Superior, and in other waters (Table 8) are not strictly comparable because of differences in gear used in collection, in the types of length measurements and dates of collection in the several studies, and because both actual and calculated lengths are included. Despite these difficulties, the data show that the rate of growth in Keweenaw Bay was markedly faster than in Great Bear Lake, Northwest Territory, Canada, slower than in Moosehead Lake, Maine, and roughly similar to growth of lake trout in other inland waters that have been studied. In Great Lakes waters the growth rate in Lake Superior was decidedly slower than in South Bay, Lake Huron, and somewhat slower than in Lake Michigan.

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[5] Cable (1956) and Van Oosten and Eschmeyer (1956) concluded that there were no important differences in the growth rate of lake trout in Lake Michigan in different calendar years.

Table 8.—Growth of lake trout in Keweenaw Bay, Lake Superior, and in other waters

| Water, and reference | Year of life completed | | | Type of length measurement |
|---|---|---|---|---|
| | 1 | 2 | 3 | |
| Great Bear Lake, N. W. T. (Miller and Kennedy, 1948) | 2.5 | 3.9 | 5.3 | Fork length at collection* |
| Pyramid Lake, Alberta (Rawson and Elsey, 1950) | ... | 6.4 | 8.9 | Fork length at collection* |
| Lake Louisa, Ontario (Martin, 1952) | 4.1 | 6.9 | 7.7 | Calculated fork length |
| Redrock Lake, Ontario (Martin, 1952) | ... | 5.8 | 7.4 | Calculated fork length |
| Brewer Lake, Ontario (Martin, 1952) | ... | 5.5 | 9.5 | Calculated fork length |
| Moosehead Lake, Maine (Cooper and Fuller, 1945) | ... | 10.0 | 11.0 | Total length at collection* |
| South Bay, Lake Huron, Ontario (Fry, 1953) | ... | 10.0 | 12.7 | Fork length at collection* |
| Montague, Lake Michigan (Van Oosten, 1950) | 4.9 | 8.5 | 11.9 | Calculated total length |
| Lake Michigan (Van Oosten and Eschmeyer, 1956) | 3.4 | 7.1 | 10.1 | Calculated total length |
| Lake Michigan (Cable, 1956) | 5.9 | 8.7 | 11.2 | Calculated total length** |
| Keweenaw Bay, Lake Superior (Present study) | 3.9 | 6.7 | 8.6 | Total length at collection† |

* Lengths may include varying amounts of growth during the season of collection.

** Hatchery-reared, fin-clipped lake trout stocked in September of their first year of life and later recovered.

† Collected in May 1953 before new growth was detected by scale examination.

## SEX RATIO

Males and females were about equally represented among 515 small lake trout (all sexually immature) collected in Lake Superior in 1952 and 1953 (Table 9). Although the percentage of males was as low as 43 among

Table 9.—Sex ratio of small lake trout, Lake Superior, 1952-1953

| Age group | Locality* | Number of fish | Number of males | Number of females | Percentage males |
|---|---|---|---|---|---|
| 0** | Laughing Fish Point (10) | 65 | 32 | 33 | 49 |
| I | Keweenaw Bay (5, 6, 7, 8) | 152 | 65 | 87 | 43 |
| II | Keweenaw Bay (6, 7, 8, 9) | 197 | 98 | 99 | 50 |
| II | Isle Royale (2, 3) | 101 | 56 | 45 | 55 |
| All fish | | 515 | 251 | 264 | 49 |

* Station numbers in parentheses.

** Data confined to collections at Laughing Fish Point on October 5 and 21-22.

yearlings caught in Keweenaw Bay and as high as 55 in collections of 2-year-old fish from Isle Royale, the data do not suggest a significant deviation fom a 50:50 ratio.

## Food

Stomach contents were examined from 325 O-group lake trout caught near Laughing Fish Point and 392 I- and II-group fish collected in 1952–1953 at stations in Keweenaw Bay and near Isle Royale. Only stomachs containing food were considered—96 percent of the young of the year, 94 percent of the yearlings, and 83 percent of the 2-year-old fish. Because contents of individual stomachs were often too small for satisfactory measurement, a single volume determination (by liquid displacement in a sedimentation tube graduated to 0.01 cubic centimeter) was made for the aggregate of each food item from an entire collection.

*Food of young of the year.*—Crustacea occurred in 93 percent of the stomachs of first-summer lake trout and composed 87 percent of the food volume (Table 10). The opossum shrimp, *Mysis relicta,* was by far the most important. It was found in fish from all collections, in 70 percent of the stomachs, and made up 84 percent of the total food. Insects occurred in 27 percent of the stomachs, but constituted only a negligible proportion of the food, whereas fish were in only 9 percent of the stomachs, but contributed 13 percent of the volume.

The above totals for all collections describe reasonably well the general food habits of lake trout collected in their first year of life, although some minor seasonal differences, perhaps associated with increasing size of the fish, were observed. In the earliest 1953 collection (June 22–23) insects formed a far more important part of the diet than in any later collection; they occurred in 80 percent of the stomachs and made up 78 percent of the food volume. Nearly all that were identifiable were pupae of phantom midges (*Chaoborus*). Their chitinous remains often formed a dense mass in the stomach immediately anterior to the duodenum. Remnants of as many as 14 pupae occurred in a single stomach.[6] Copepods also were important in the diet of fish of this collection (in 44 percent of the stomachs and comprised 11 percent of the volume). A single mysid, which caused a marked distention of the stomach of the lake trout that had eaten it, also composed 11 percent of the total food volume.

*Mysis* became progressively more frequent in stomachs of young-of-the-year lake trout collected in July and August 1953,[7] and composed 76 to 96 percent of the food. Copepods continued to be numerous, although they contributed importantly to the volume only in the small collection of July 22. Insects did not occur in the July collections, but were in 28 and 41 percent of the stomachs in the two large August collections. On August 12 the insects were principally terrestrial forms that were probably taken at or near the lake bottom; on August 24 phantom-midge pupae were again the principal insect. Fish (two ninespine sticklebacks, one cottid, and several tiny unidentified fry) were first observed in the stomachs from August collections.

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[6] Larval forms of aquatic insects were rare in stomachs of O-group lake trout (in only 6 of the 325 stomachs examined; not more than 2 occurred in any one stomach).

[7] Food in the stomachs of 30 young-of-the-year lake trout caught near Laughing Fish Point on August 27, 1952, was closely similar to that in fish of the August 1953 collections. Therefore, these records were included in Table 10.

**Table 10.—Percentage frequency of occurrence and (in parentheses) percentage of total volume composed by food items in stomachs of O-group lake trout, Laughing Fish Point, Lake Superior, 1952–1953**

[All collection dates were in 1953 except for the collection of August 27, 1952. Tr indicates volume or number less than 0.5 percent. Taxonomic groups in footnotes show principal components of higher groups]

| Item | June 22–23 | July 13 | July 22 | Aug. 12 | Aug. 24 | Aug. 27, 1952 | September 14 | | Oct. 5 | Oct. 21–22 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | (13–23 fathoms) | (26–39 fathoms) | | | |
| Total length (inches) | 1.1–1.3 | 1.2–1.4 | 1.3–1.5 | 1.5–2.1 | 1.7–2.6 | 1.6–2.1 | 2.3–3.2 | 2.2–3.0 | 2.1–3.5 | 2.6–3.7 | 1.1–3.7 |
| Number of stomachs | 25 | 10 | 8 | 47 | 73 | 30 | 46 | 23 | 31 | 32 | 325 |
| Food item: | | | | | | | | | | | |
| Crustacea | 48 | 100 | 100 | 96 | 96 | 100 | 100 | 87 | 97 | 97 | 93 |
| | (22) | (100) | (100) | (94) | (87) | (94) | (48) | (100) | (71) | (98) | (87) |
| Cladocera* | .. | .. | .. | .. | 4 | .. | 96 | 9 | .. | .. | 15 |
| | | | | | (tr) | | (19) | (tr) | | | (1) |
| Copepoda** | 44 | 60 | 88 | 49 | 7 | .. | 100 | 9 | .. | .. | 31 |
| | (11) | (4) | (24) | (4) | (tr) | | (29) | (tr) | | | (2) |
| Ostracoda | .. | .. | .. | .. | 3 | .. | 9 | 4 | .. | .. | 2 |
| | | | | | (tr) | | (tr) | (tr) | | | (tr) |
| Mysidacea† | 4 | 50 | 62 | 85 | 88 | 100 | 2 | 83 | 97 | 97 | 70 |
| | (11) | (96) | (76) | (90) | (87) | (94) | (tr) | (100) | (71) | (98) | (84) |
| Amphipoda†† | .. | .. | .. | .. | .. | .. | .. | .. | .. | 3 | tr |
| | | | | | | | | | | (tr) | (tr) |
| Hydracarina | 8 | .. | .. | 2 | .. | .. | .. | .. | .. | .. | 1 |
| | (tr) | | | (tr) | | | | | | | (tr) |
| Insecta‡ | 80 | .. | .. | 28 | 41 | 17 | 20 | 13 | 19 | 3 | 27 |
| | (78) | | | (tr) | (1) | (1) | (1) | (tr) | (tr) | (tr) | (tr) |
| Pupae and larvae‡‡ | 60 | .. | .. | 4 | 37 | 17 | 20 | 4 | 3 | .. | 18 |
| | (78) | | | (tr) | (1) | (1) | (1) | (tr) | (tr) | | (tr) |
| Adult§ | 12 | .. | .. | 15 | 4 | .. | .. | 9 | 16 | 3 | 10 |
| | (tr) | | | (tr) | (tr) | | | (tr) | (tr) | (tr) | (tr) |
| Pelecypoda§§ | .. | .. | .. | .. | .. | .. | .. | .. | .. | 3 | tr |
| | | | | | | | | | | (tr) | (tr) |
| Fish# | .. | .. | .. | 6 | 20 | 10 | 6 | .. | 16 | 3 | 9 |
| | | | | (6) | (12) | (5) | (51) | | (29) | (2) | (13) |

* *Leptodora, Daphnia.* ** *Epischura, Diaptomus, Cyclops.* † *Mysis.* ††*Pontoporeia.* ‡ Includes fragments of unidentified insects not included in other categories shown. ‡‡ *Chaoborus, Chironomus.* § Diptera, Hymenoptera, Coleoptera. §§ Sphaeriidae. # Slimy sculpins, unidentified cottids, and ninespine sticklebacks.

The collection of September 14, 1953, (following the severe storm of September 12–13) revealed an unusual relationship between the food eaten and the depth of collection. Of 46 stomachs from shallow water (13–23 fathoms), 41 contained *Leptodora* (this was the only date on which it was found in lake trout stomachs), whereas none of 23 fish collected at greater depths (26–39 fathoms) had eaten this organism. Copepods occurred in all fish from the shallower water, but in only two from greater depths. *Mysis,* established by earlier and later collections as predominant in the diet, occurred in only 1 stomach of fish from the shallower water, but in 19 (83 percent) from the deeper water. Insect remains were common in stomachs from both deep and shallow water. Fish (slimy sculpins and unidentified Cottidae) were in only the shallow-water collection, where they composed more than half of the total food. Although the average volume of food (all items combined) in stomachs of the collections from different depths was closely similar, its composition was extremely different. Unquestionably, the storm of September 12–13 and the subsequent unusual bathymetric distribution of O-group trout (Table 2) exerted a marked effect on their diet. The occurrence of *Mysis* in only the collection from deep water adds support to the earlier suggestion that large masses of water were moved during the storm. Mysids may have been carried out of the shallower waters which they normally occupied and thus were not available to lake trout at these depths.

*Mysis* dominated the diet of young-of-the-year lake trout caught in October. They were in 97 percent of the stomachs and composed 71 to 98 percent of the food. Fish (slimy sculpins and other Cottidae not further identified) made up the remainder of the food volume. Fragments of insects (mostly terrestrial adults), amphipods, and one small sphaeriid clam also were present.

Small quantities of very finely divided organic detritus (not listed in Table 10) occurred in 5 percent of the stomachs. Small stones—as many as eight in a single stomach—were found in 12 percent of the trout. On September 14, stones occurred in 15 percent of the fish from shallow water, and in 48 percent of those from the deeper strata. Of stones ingested by lake trout at the greater depths on that date, many were flat, shiny, micaceous fragments which may have been suspended above the bottom by water movements during the preceding storm and mistakenly seized by the lake trout as food. Young-of-the-year lake trout collected at other stations had eaten foods similar to those taken by fish from Laughing Fish Point. In the large sample collected from Keweenaw Bay on August 24–25, 1952, (Table 1), the food was almost entirely *Mysis,* which occurred in all of the 55 stomachs and composed 99 percent of the food. Insects and fish each occurred in one stomach. Copepods (as many as 192 in a single stomach), mysids, phantom-midge pupae, and fish occurred in stomachs of the July 29, 1953, collection of nine lake trout from the Apostle Islands (Station 4). A 1.5-inch lake trout of this collection was the smallest that contained fish (tiny, unidentified larvae).

Only limited records have been published on the food of lake trout during the first year of life. Kendall and Goldsborough (1908) found blackfly larvae, mosquitoes, insect fragments, and insect eggs in 6 lake trout (1.9 to 2.4 inches long) collected in a tributary of First Lake, Connecticut, in July and August. Neave and Bajkov (1929) reported chironomid larvae and small adult Diptera in 10 trout (1.3–1.8 inches long) from an inlet creek of Pyramid Lake, Alberta.

*Food of I- and II-group lake trout.*—Despite some differences the food habits of yearling and 2-year-old lake trout were sufficiently similar to justify the combination of data for the two groups. Collections from Keweenaw Bay and from stations near Isle Royale (to which this part of the food study was confined) showed similar stomach contents; therefore records from the two localities also were combined.

The food of I- and II-group lake trout was similar to that of O-group lake trout (Table 11). Crustacea were found in 98 percent of the stomachs and composed 88 percent of the food volume (as compared to 93 and 87 percent, respectively, among O-group fish). *Mysis* occurred in 95 percent of the stomachs and made up 82 percent of the food. The amphipod, *Pontoporeia,* was the only other crustacean found in more than 1 percent of the fish. It was in 5 percent of the stomachs and made up 5 percent of the food. *Pontoporeia* contributed importantly to the diet only in the July collections from Keweenaw Bay, when it was in stomachs of 24 percent of the yearlings and 50 percent of the 2-year-old fish and made up 28 and 69 percent, respectively, of the food. Amphipods did not occur in collections from Isle Royale.

**Table 11.—Percentage frequency of occurrence and (in parentheses) percentage of total volume composed by food items in stomachs of I- and II-group lake trout, Keweenaw Bay and Isle Royale, Lake Superior, 1952–1953**

[All collection dates were in 1953 except the collection of August 25–26, 1952. Tr indicates volume less than 0.5 percent. Taxonomic groups in footnotes show principal components of higher groups]

| Item | May 7–12 | July 8–26 | Aug. 25–26, 1952 Aug. 2, 1953 | Sept. 3–11 | Sept. 29–Oct. 16 | Total |
|---|---|---|---|---|---|---|
| Total length (inches) | 3.6–7.4 | 4.2–7.7 | 4.3–8.2 | 4.3–8.3 | 4.6–8.9 | 3.6–8.9 |
| Number of stomachs | 37 | 58 | 49 | 111 | 137 | 392 |
| Food item | | | | | | |
| Crustacea | 97<br>(94) | 100<br>(100) | 100<br>(87) | 95<br>(82) | 98<br>(86) | 98<br>(88) |
| Cladocera* | 3<br>(tr) | .. | .. | 1<br>(tr) | 1<br>(tr) | 1<br>(tr) |
| Copepoda | 5<br>(tr) | .. | .. | .. | .. | 1<br>(tr) |
| Ostracoda | .. | .. | .. | 2<br>(tr) | .. | 1<br>(tr) |
| Mysidacea** | 92<br>(94) | 88<br>(61) | 100<br>(87) | 95<br>(82) | 98<br>(86) | 95<br>(82) |
| Amphipoda† | .. | 26<br>(39) | 2<br>(tr) | 4<br>(tr) | 1<br>(tr) | 5<br>(5) |
| Insecta†† | 3<br>(tr) | 19<br>(tr) | 18<br>(tr) | 31<br>(1) | 22<br>(tr) | 22<br>(tr) |
| Pupae and larvae‡ | .. | 10<br>(tr) | 8<br>(tr) | 11<br>(tr) | 8<br>(tr) | 8<br>(tr) |
| Adult‡‡ | 3<br>(tr) | 9<br>(tr) | 12<br>(tr) | 20<br>(1) | 9<br>(tr) | 12<br>(tr) |
| Fish§ | 3<br>(6) | .. | 12<br>(13) | 9<br>(17) | 13<br>(14) | 9<br>(12) |

* *Daphnia.* ** *Mysis.* † *Pontoporeia.* †† Includes fragments of unidentified insects not included in other categories shown. ‡ *Chaoborus, Chironomus.* ‡‡ Diptera, Hymenoptera, Coleoptera, Trichoptera. § Cottidae (including a slimy sculpin), coregonines (including pygmy whitefish), and smelt.

Insects were in 22 percent of the stomachs (as compared to 27 percent in the O group), but made up less than 0.5 percent of the volume. Pupae of phantom midges were again the most important form; larval aquatic insects were in only 7 of the 392 fish. Adult terrestrial insects occurred in 12 percent of the stomachs.

Fish were found in 9 percent of the stomachs and made up 12 percent of the volume of food in I- and II-group lake trout (compared to 9 and 13 percent, respectively, in the O-group—Table 10). Identifiable remains included 10 Cottidae (7 of the genus *Cottus,* 1 of which was further identified as a slimy sculpin), 5 coregonines (3 pygmy whitefish,[8] 2 not further identifiable), and 1 smelt.

The relative importance of fish in the diet was the major difference among the age groups and regional collections for which data were combined in Table 11. Fish were found in 18 percent of the stomachs and made up 25 percent of the food of 166 II-group lake trout from Keweenaw Bay; they occurred in only 4 percent of the stomachs and made up only 1 percent of the food in 142 yearlings from this region; and they did not occur in stomachs of 81 2-year-olds and 3 yearlings from Isle Royale.

Although fish may be important as food in lake trout of some collections, it is clear that *Mysis* was by far the most important item in the diet during the first three years of life of lake trout of Lake Superior.

Martin (1952) found plankton the most important food in 80 lake trout from Lake Louisa and 16 from Redrock Lake, Ontario (the fish were 5 to 9 inches, fork length). Bottom organisms and fish were also present. (In Lake Louisa, plankton made up a sizable proportion of the diet of even larger lake trout, 15 or more inches long, during the summer.)

Royce (1951) found 9 to 34 *Mysis* in each of nine lake trout, 6.5–7.9 inches long, collected September 13–16, 1940, in Keuka Lake, New York. One of these fish had eaten a slimy sculpin. Rimsky-Korsakoff (1930) reported midge larvae, adult insects, and plankton in three lake trout, 4.9–6.8 inches in standard length, from the Champlain Watershed, New York. The stomachs of three lake trout, 7.9 to 9.1 inches long, from Lake Nipigon contained ciscoes (Clemens *et al.,* 1923).

In Great Lakes waters, Van Oosten and Deason (1938) examined stomachs of 4,386 lake trout collected in Lake Michigan in 1931–1932, but only 12 of these were less than 10 inches long. Cottidae and invertebrates predominated in stomachs of lake trout less than 15 inches in length. Among the invertebrates, *Mysis* occurred most frequently.

In most other published studies of the food of lake trout, the results of analyses of food of the smaller fish were not separated from data for larger individuals. It seems clear, however, that in most waters lake trout less than 9 inches long feed primarily on invertebrates, whereas fish compose a varying, but less important, proportion of the diet.

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[8] The pygmy whitefish has become known only recently in the Lake Superior fauna. Its occurrence there and details of its life history were reported by Eschmeyer and Bailey (1955).

## SPECIES ASSOCIATED WITH SMALL LAKE TROUT

Sixteen species or groups of species (the latter not specifically identified) were collected with O-group and 15 with I- and II-group lake trout. Estimates of the "degree of association" have been made in terms of percentage occurrence in trawl hauls that took lake trout of these age groups and percentage occurrence at stations (and on the same dates and depths) at which lake trout were caught (Tables 12 and 13).

The most common associates of young-of-the-year lake trout were cottids, which occurred in 94 percent of the hauls and at all of the stations where the young were collected (Table 12). Slimy sculpins were taken most often; fourhorn sculpins occurred in numbers only in the deeper waters. Ninespine sticklebacks came next in importance, followed by smelt and unidentified ciscoes. Pygmy whitefish, trout-perch, shallowwater ciscoes and older lake trout were other relatively important associates of first-summer lake trout, but the remainder of the species listed occurred in only 5 percent or less of the hauls and at only one or two stations.

Cottidae, ninespine sticklebacks, and smelt were the most common associates of 1- and 2-year-old lake trout (Table 13), as they were of the O group. These were followed by pygmy whitefish, chubs, older lake trout, shallowwater ciscoes, unidentified ciscoes, trout-perch and burbot. The other species listed were less common associates. Four species occasionally found with O-group lake trout in shallow water—log perch, round whitefish, yellow perch, and johnny darters—were not caught in trawl hauls or at stations which yielded I- or II-group lake trout. Three species, the lake whitefish, brook stickleback, and mudminnow, were caught with the older trout, but not with O-group fish. The brook stickleback and mudminnow were caught at depths of 43–48 fathoms in southern

**Table 12.—Associates of O-group lake trout in Lake Superior, 1953**

[The ranking of each species or group of species is expressed in terms of its percentage frequency of occurrence in 80 trawl hauls and at 8 stations where O-group lake trout were caught]

| Species | Percentage frequency of occurrence | |
|---|---|---|
| | In individual hauls | At stations |
| Cottids* | 94 | 100 |
| Ninespine stickleback | 58 | 75 |
| American smelt | 42 | 62 |
| Unidentified ciscoes** | 15 | 62 |
| Pygmy whitefish | 24 | 50 |
| Trout-perch | 18 | 50 |
| Shallowwater cisco | 12 | 50 |
| Older lake trout | 10 | 25 |
| Burbot | 5 | 25 |
| Ciscoes** | 5 | 25 |
| White sucker | 2 | 25 |
| Logperch | 2 | 25 |
| Longnose sucker | 2 | 12 |
| Round whitefish | 1 | 12 |
| Yellow perch | 1 | 12 |
| Johnny darter | 0 | 12 |

* Slimy, spoonhead, and fourhorn sculpins.
** Bloater, shortjaw, and shortnose ciscoes.

**Table 13.—Associates of I- and II-group lake trout in Lake Superior, 1953**

[The ranking of each species or group of species is expressed in terms of its percentage frequency of occurrence in 61 trawl hauls and at 8 stations where I- and/or II-group lake trout were caught]

| Species | Percentage frequency of occurrence | |
|---|---|---|
| | In individual hauls | At stations |
| Cottids* | 90 | 75 |
| Ninespine stickleback | 82 | 75 |
| American smelt | 69 | 88 |
| Pygmy whitefish | 62 | 50 |
| Ciscoes** | 49 | 62 |
| Older lake trout | 46 | 62 |
| Shallowwater cisco | 25 | 62 |
| Unidentified ciscoes** | 44 | 38 |
| Trout-perch | 41 | 38 |
| Burbot | 23 | 38 |
| O-group lake trout | 11 | 25 |
| Longnose sucker | 18 | 12 |
| White sucker | 5 | 25 |
| Lake whitefish | 8 | 12 |
| Brook stickleback | 2 | 12 |
| Mudminnow | 1 | 12 |

* Slimy, spoonhead, and fourhorn sculpins.
** Bloater, shortjaw, and shortnose ciscoes, and possibly the kiyi.

Keweenaw Bay (Station 7) on May 12, 1953. This occurrence in deep water is unusual as these species are most commonly found in much shallower habitats.

No species in addition to those taken in trawls were collected in gill nets that caught 2-year-old lake trout. Ciscoes, older lake trout, smelt, and burbot were commonly taken in gangs of experimental gill nets which caught 2-year-old lake trout.

## SUMMARY

Young-of-the-year, yearling, and 2-year-old lake trout were collected in bottom trawls by the U. S. Fish and Wildlife Service research vessel *Cisco* in Lake Superior in 1952 and 1953. Trawl collections of 2-year-old trout were supplemented by catches in gangs of experimental nylon gill nets of graded mesh.

Young-of-the-year lake trout were collected at eight stations in southern Lake Superior from the Apostle Islands, Wisconsin, eastward to Grand Marais, Michigan. Most were caught off Laughing Fish Point about 20 miles east of Marquette. Collections in June, July, and August were principally from depths less than 20 fathoms. After a severe storm in mid-September, first-summer lake trout were distributed over a wide range of depths. In October most were caught in water more than 20 fathoms deep. Water temperatures at depths and on dates of collection of young-of-the-year lake trout off Laughing Fish Point ranged from 42° to 63°F.

Most I- and II-group lake trout taken in trawls were caught in Keweenaw Bay at depths of 20–34 fathoms. Gill-net catches here and in other areas supported trawling results in showing the relatively greater abundance of II-group lake trout in the 20–34-fathom interval than at other depths. At these depths temperatures ranged from 39° to 53°F. in Keweenaw Bay and from 39° to 43°F. at stations near Isle Royale, on dates when small lake trout were collected.

Young-of-the-year lake trout in the 1953 collections near Laughing Fish Point increased in mean total length from 1.2 inches on June 22–23 to 3.1 inches on October 21–22. The greatest rate of increase (4 millimeters per week) occurred between July 22 and September 14. Yearlings in Keweenaw Bay were 3.9 inches long on May 11–12 and 5.6 inches on October 15; for 2-year-old fish these lengths were 6.7 and 8.0 inches respectively. Lake trout of the II group collected near Isle Royale were markedly shorter than fish of the same age collected on similar dates in Keweenaw Bay. A comparison of length of lake trout in October with length in May, before new growth had occurred, strongly suggests that the growing season of small lake trout continues to or beyond the end of the calendar year. The average lengths of 1-, 2-, and 3-year-old lake trout caught in Keweenaw Bay in May 1953 were 3.9, 6.7, and 8.6 inches, respectively.

The opossum shrimp (*Mysis*) was by far the most important food of small lake trout from Lake Superior. It was in 70 percent of the stomachs and made up 84 percent of the food volume in 325 young-of-the-year lake trout; for 392 I- and II-group fish these percentages were 95 and 82, respectively. Other crustacea, insects, and fish were less important in the diet.

Cottids, ninespine sticklebacks, smelt, coregonines, trout-perch, and older lake trout were the most common associates of lake trout during their first three summers of life in Lake Superior.

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# A NEW SPECIES OF CANDACIA (COPEPODA: CALANOIDA) FROM THE WESTERN NORTH ATLANTIC OCEAN

By **Abraham Fleminger** and **Thomas E. Bowman**

The new species of *Candacia* described below was discovered independently by the authors while engaged in studies of plankton collections made by the U. S. Fish and Wildlife Service (USFWS). The plankton tows were made off the southern Atlantic coast of the United States by the *Theodore N. Gill* and in the Gulf of Mexico by the *Alaska* as part of general oceanographic surveys of the two regions.

In addition to the new species described below, seven known species of *Candacia* have been found in the collections. *C. bipinnata* Giesbrecht, *bispinosa* Claus, *curta* Dana, *longimana* Claus, *pachydactyla* Dana, and *simplex* Giesbrecht occurred in both regions surveyed, whereas *armata* Boeck has been found only in collections made at some of the most northern stations occupied by the *Gill*, off Beaufort, N. C. These stations appear to represent approximately the southern boundary of the distribution of this species along the Atlantic coast of the United States. As pointed out by Thompson and Scott (1903, p. 250), Brady's (1884, p. 68) records from *Challenger* stations are probably erroneous. The only other published records from Indo-Pacific or tropical western Atlantic localities are contained in C. B. Wilson's *Carnegie* (1942) and *Albatross* (1950) reports. Examination of *Carnegie* and *Albatross* specimens in the U. S. National Museum

(USNM) identified by Wilson as *C. armata* was carried out. Almost all of Wilson's determinations proved to be erroneous; *C. armata* was not present at any of the *Carnegie* stations and only at *Albatross* station 2195 (lat. 39°44′ N., long. 70°03′ W., off Cape Hatteras).

*C. aethiopica* Dana, a widespread species in temperate and tropical waters of all the oceans, was not found in either the *Alaska* or *Gill* collections. It will probably be found at least in the region covered by the *Gill*, since it has been reported from the Bermuda area (Moore, 1949), and Mr. Philip St. John (personal communication) has found it in plankton hauls made off the coast of North Carolina.

*Candacia norvegica* (Boeck), found in the cooler part of the North Atlantic Ocean, appears to be limited to waters of lower temperature than those surveyed by the *Alaska* and the *Gill*. In Wilson's *Carnegie* and *Albatross* papers this species was reported to have been found in the Sargasso and Caribbean regions and in many parts of the Pacific Ocean. As in the case of *C. armata*, Wilson's determinations have been found to be incorrect. *C. norvegica* was not present in any of the *Albatross* or *Carnegie* material in the U. S. National Museum.

### ***Candacia paenelongimana*, new species**

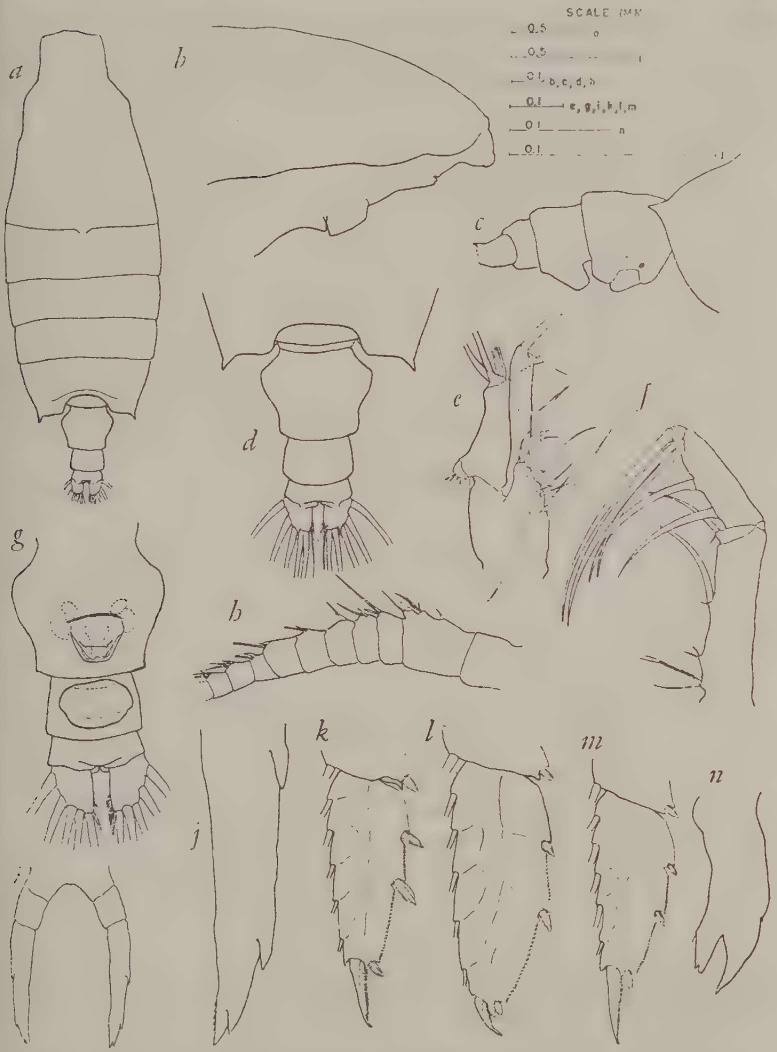
FIGURES 1; 2,*a–g*

LOCALITIES AND MATERIAL: Gulf of Mexico: Lat. 23°35′ N., long. 82°23′ W. (USFWS *Alaska* cruise 4, station 29, Jan. 19, 1952, 1 meter depth of tow, one female); lat. 23°31′ N., long. 86°44′ W. (*Alaska* cruise 4, station 36, Jan. 24, 1952, 1 meter depth of tow, one female).

Gulf Stream: Off Florida coast, lat. 27°40′ N., long. 79°41′ W. (USFWS *Theodore N. Gill* cruise 1, station 6, Feb. 17, 1953, 60 meters depth of tow, one female); off South Carolina coast, lat. 32°41′ N., long. 77°03′ W. (*Gill* cruise 1, station 62, Mar. 3, 1953, 75 meters depth of tow, one female, one male).

MEASUREMENTS: All measurements made from dorsal view along midsagittal plane; cephalothorax measured from anteriormost margin of forehead to posterior margin of intersegmental fold between thoracic fusion segment IV–V and genital segment; length of abdomen from anterior margin of genital segment to articulation between fifth innermost seta and right furcal ramus. Measurements made at 100× magnification with aid of ocular micrometer, specimens immersed in solution of 50 percent glycerine, 50 percent water. Slender glass rods used to support cephalothorax and abdomen in horizontal position during measurements of each.

The total length and cephalothorax-abdomen ratio, presented in that order, for individuals collected at different localities are as follows: Adult females: holotype, 2.92 mm., 4.1 : 1; paratypes, 2.54


a
b
SCALE (MM)
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0.1 b, c, d, h
0.1 e, g, i, k, l, m
0.1 n
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c
d
e
f
g
h
i
j
k
l
m
n

FIGURE 1.—*Candacia paenelongimana* new species, female (*a-j* and *n* are holotype, *k-m*, paratype): *a*, dorsal view; *b*, cephalon, lateral view; *c*, abdomen, lateral view; *d*, abdomen, dorsal view; *e*, second maxilla; *f*, maxilliped; *g*, abdomen, ventral view; *h*, proximal portion of first antenna; *i*, fifth legs; *j*, ramus of fifth leg, distal half; *k*, third exopodal segment of fourth leg; *l*, third exopodal segment of third leg; *m*, third exopodal segment of second leg; *n*, mandible, gnathal lobe.

mm., 3.9 : 1 (*Alaska* station 29); 2.57 mm., 3.7 : 1 (*Gill* station 62) 2.66 mm. (*Gill* station 6). Adult male: allotype, 2.49 mm., 3.4 : 1.

Diagnosis: Adult female; similar to *C. longimana* (Claus) with respect to cephalothorax, cephalic appendages, and swimming legs 1–4 (fig. 1,*e,f,h,k–n*).

Abdominal details differing from *longimana*: Genital segment with somewhat angular lateral swellings, greatest width anterior to midlength of swellings (fig. 1,*d,g*); measured at greatest width, genital segment wider than long (0.82–0.85 : 1); second abdominal segment produced ventrad in a broad lobiform process (fig. 1,*c*); process extends ventrad and somewhat anteriad, almost reaching posteroventral margin of genital segment.

Fifth legs differing from *longimana*; spines of ramal segment small; ramus with two apical subequal spines, two appressed spines along lateral margin; medial margin of ramus with longitudinal row of about five small denticles near apex (fig. 1,*i,j*).

Adult male similar to *longimana* with respect to cephalic appendages and swimming legs 1–5.

Posterior process of right fifth thoracic segment relatively longer and of rather different form than that of *longimana*. Seen from above (fig. 2,*c*) process tapers gradually and turns slightly outward at tip; in lateral view (fig. 2,*b*) it curves upward to tip, which has an expansion on its posterior side.

Genital segment (fig. 2,*a,d*) similar to that of *longimana*, but process on right side without thickening near the middle as characteristic of *longimana*.

Fifth legs (fig. 2,*e*) like those of *longimana* in all details.

Types: Holotype, USNM 98618, female, *Alaska* cruise 4, station 36. Allotype, USNM 98619, male, *Gill* cruise 1, station 62. Paratypes: USNM 98622, female, *Alaska* cruise 4, station 29; USNM 98621, female, *Gill* cruise 1, station 6; USNM 98620, female, *Gill* cruise 1, station 62.

Additional Description: Abdominal segments plus furca with following proportions: Female (based on holotype), 48.5, 25.0, 12.0, 14.5=100; male, 37.5, 30.0, 27.0, 12.5, 20.0=100.

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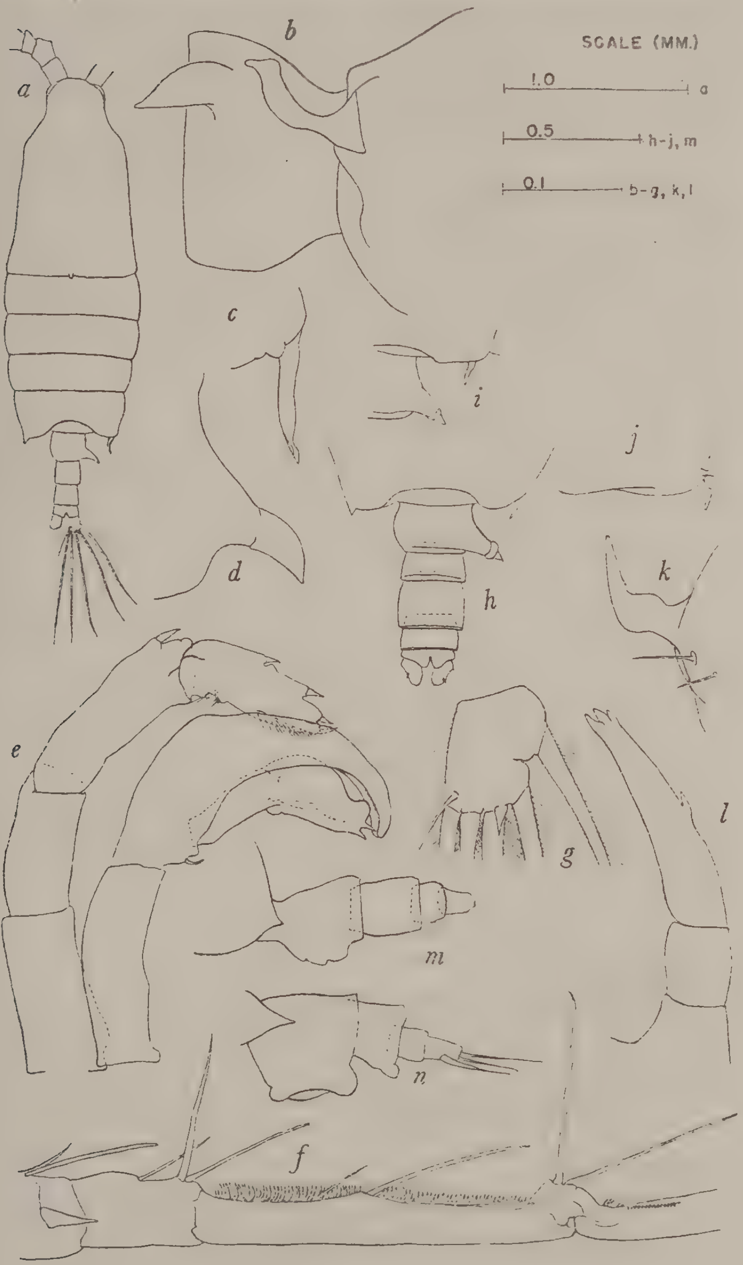
Figure 2.—*a-g, Candacia paenelongimana* new species, male allotype: *a*, dorsal view; *b*, fifth thoracic segment and first abdominal segment, seen from the right side; *c*, posterior corner of right fifth thoracic segment, dorsal view; *d*, process of first abdominal segment, dorsal view; *e*, fifth legs; *f*, right first antenna, segments 15–19, dorsal view; *g*, right furcal ramus, dorsal view. *h-l, Candacia longimana* (Claus): *h*, abdomen, dorsal view, male from South Pacific, USNM 80116; *i*, portion of fifth thoracic and first abdominal segments, dorsal view, male from same lot as *h*; *j*, posterior corner of right fifth thoracic segment, dorsal view, male from *Carnegie* Sta. 66, off Chile, USNM 80115; *k*, same, lateral view; *l*, fifth legs, female, *Gill* Cruise 1, Sta. 71, off North Carolina; *m*, abdomen, lateral view, same specimen. *n, Candacia chirura* Cleve, abdomen, lateral view, from Farran (1929).


a
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e
f
g
h
i
j
k
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m
n
SCALE (MM.)
1.0 a
0.5 h-j, m
0.1 b-g, k, l

FIGURE 2.—Explanation on facing page.

Cephalothorax-abdomen length ratio of female *longimana* Claus, according to Giesbrecht (1892, p. 428), approximates 2.9±.3:1. However, ratios of *longimanna* collected by *Gill* and *Alaska* similar to range in *paenelongimana*; a female *longimana* from Gulf of Mexico (unpublished data; lat. 23°50′ N., long. 82°19′ W., Jan. 19, 1952, 1 meter depth) with ratio of 3.8:1; two *longimana* females from off Cape Lookout (unpublished data; lat. 34°03′ N., long. 75°15′ W., Mar. 6, 1953, 35 meters depth) with ratios of 3.6:1 and 4.1:1. Cephalothorax-abdomen length ratio, therefore, appears to be of no value in distinguishing between individual females of the two species.

Genital segment tends to be longer and furcal rami shorter in female *paenelongimana* as compared to available specimens and Giesbrecht's figures (1892, pl. 39, figs. 4, 6) of *longimana*.

Remarks: The female can be easily recognized by the characteristic ventral process of the second abdominal segment. While processes from the vental surface of this segment are developed to varying degrees in several species of *Candacia*, the process is directed anteriad only in *paenelongimana*. Both *Candacia chirura* Cleve (fig. 2,*n*) and *C. armata* (Boeck) have conspicuous processes, directed posteriad.

The male can easily be confused with the male of *longimana*, but can be distinguished by the shape of the process on the right side of the fifth thoracic segment, especially when examined from the side. In *longimana* this process is somewhat variable, but is relatively shorter and heavier than in the single male *paenelongimana*. In dorsal view (fig. 2,*h–j*) it is more pointed and usually curves medially. Seen laterally (fig. 2,*k*) it bends abruptly upward just beyond the thickened middle and narrows uniformly to the pointed distal end. A number of male specimens of *longimana* from both the Atlantic and Pacific Oceans have been examined; in none of them did this process closely resemble that of *paenelongimana* with its uniform curvature and expanded apex.

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# FURTHER OBSERVATIONS ON STREAM SURVIVAL OF KING SALMON SPAWN[1]

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## INTRODUCTION

An experimental hatching study of king salmon (*Oncorhynchus tshawytscha*) eggs in Mill Creek, a tributary of the Sacramento River, California, in the fall of 1952 suggested a direct relationship between floods and egg mortalities (Gangmark and Broad, 1955). This study was a part of a general investigation of the reasons for widely fluctuating survival rates of salmon from year to year. Further information on the influence of floods being desirable, additional investigations were carried out during the 1953-1954 season as a part of the more general investigation at Mill Creek.

In the experiments at Mill Creek incubating king salmon eggs were observed in their natural environment in order to determine the effect of various influences on survival of eggs near the southernmost limit of the spawning range of the species. Fertilized eggs from native king salmon were placed in containers which were buried in spawning riffles and covered with gravel to simulate natural conditions as closely as possible. One-fifth of the containers were removed from the stream bed at equal intervals during the incubation period. Laboratory examination of the contents of each container was then made. The results were tabulated and related to various ecological conditions that may have occurred during incubation. During the later stages of the experiment some eggs could not be accounted for, even though the eggs were in plastic sacks. An accurate record of survival rates was kept by using samples of known size.

## THE EXPERIMENTAL AREA

Several requirements for a suitable area for the tests were met by a site near Woodward's Gate (Figure 1). It would be accessible under heavy flows, since the stream at this point could spread laterally 150 feet before it would become much deeper. The area was well supplied with gravel of the size which Burner (1951) terms as medium and small (less than six inches at its greatest breadth). Finally, spawning salmon actually had used the riffle in the past.

A controlled flow stream was also desired, to compare survival in the absence of stream fluctuation. Approximately two miles from the mouth of Mill Creek, an old channel which had become isolated from the main stream by silting and brush growth leads to the Sacramento

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[1] Submitted for publication June, 1955.

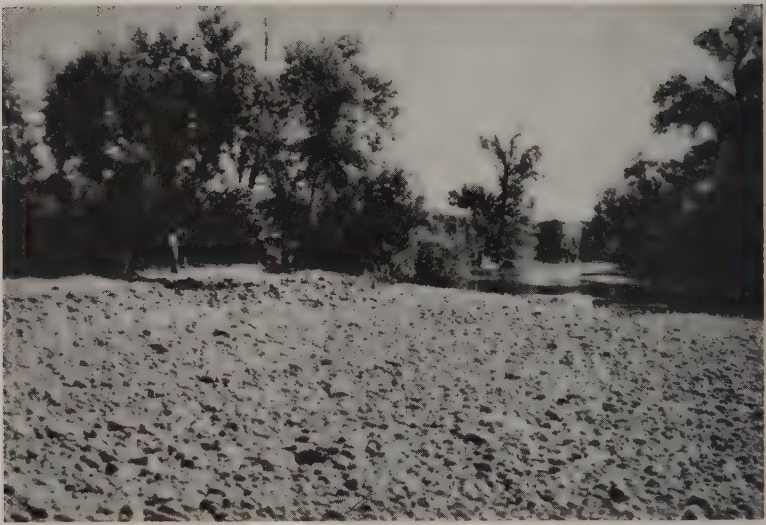

FIGURE 1. Spawning riffle area used for Mill Creek hatching experiments at Woodward's Gate in 1953-1954, exposed at low water.

River. This channel was dry except during high water, although some sections were fed by an underground spring.

Permission was obtained to divert a controlled flow into this channel, and to prevent flooding an earth fill dam was built across the entrance over a 30-inch culvert connecting the channel with Mill Creek. A headgate was installed to control water flow through the culvert.

## RESULTS OF THE FIRST WOODWARD'S GATE EXPERIMENT

On November 6, 1953, two female and four male king salmon were obtained from Mill Creek and were artifically spawned, using the hatchery dry method described by Cameron (1940). The eggs were then placed in a hatchery type egg basket in the current of the stream. After water-hardening for 1½ hours, 100 eggs were placed in each of the numbered sacks, together with small amounts of gravel. The sacks were gently set into the depressions prepared for them in the stream bed at Woodward's Gate and were covered to the level of the surrounding gravel. The planting scheme was such that each removal group would represent as much of the whole planting area as possible. Each nest was protected from spawning salmon by a piece of wire netting 2 feet square anchored over it.

The first 50 sacks contained eggs from one female, and the second 50 sacks contained eggs from the second female plus some from the first. Knowledge of the time interval required for hatching was needed in order to divide the entire trial into equal periods. Past temperature records for Mill Creek indicated that approximately 1,000 heat units would be required for hatching.

When the sacks of eggs had been buried for three days, the first group was removed to obtain information on the handling loss. The next group was removed when the accumulated heat units approximated 20 percent or 250 heat units. Other lots were removed on a similar schedule of approximately 500, 750, and 1,000 heat units. The daily temperatures and heat units during the experimental period are shown in Table 1.

After the sacks had been removed from the stream bed, the eggs were separated from the gravel and placed in bottles containing a

TABLE 1

Mill Creek Stream Hatching Experiment Heat Unit Accumulation During Winter of 1953-54

| Date | Mean daily stream temp., °F. | Heat units | Sub-totals | |
|---|---|---|---|---|
| | | | Expt. 1 | Expt. 2 |
| Nov. 6 | | 11 | x | |
| 7 | 53 | 21 | | |
| 8 | 51 | 19 | | |
| 9 | 51 | 19 | | |
| 10 | 51 | 19 | | |
| 11 | 52 | 20 | | |
| 12 | 53 | 21 | | |
| 13 | 53 | 21 | | |
| 14 | 53 | 21 | | |
| 15 | 52 | 20 | | |
| 16 | 51 | 19 | | |
| 17 | 49 | 17 | | |
| 18 | 44 | 12 | | x |
| 19 | 44 | 12 | | |
| 20 | 44 | 12 | 257 | |
| 21 | 44 | 12 | | |
| 22 | 47 | 15 | | |
| 23 | 50 | 18 | | |
| 24 | 50 | 18 | | |
| 25 | 51 | 19 | | |
| 26 | 50 | 18 | | |
| 27 | 50 | 18 | | |
| 28 | 49 | 17 | | |
| 29 | 48 | 16 | | |
| 30 | 48 | 16 | | |
| Dec. 1 | 48 | 16 | | |
| 2 | 47 | 15 | | |
| 3 | 47 | 15 | | |
| 4 | 47 | 15 | | |
| 5 | 45 | 13 | 498 | 265 |
| 6 | 45 | 13 | | |
| 7 | 45 | 13 | | |
| 8 | 45 | 13 | | |
| 9 | 45 | 13 | | |
| 10 | 45 | 13 | | |
| 11 | 44 | 12 | | |
| 12 | 43 | 11 | | |
| 13 | 43 | 11 | | |
| 14 | 45 | 13 | | |
| 15 | 46 | 14 | | |
| 16 | 46 | 14 | | |
| 17 | 47 | 15 | | |
| 18 | 48 | 16 | | |
| 19 | 49 | 17 | | |
| 20 | 50 | 18 | | |
| Dec. 21 | 47 | 15 | | |
| 22 | 44 | 12 | | |
| 23 | 43 | 11 | | |
| 24 | 42 | 10 | 755 | 519 |
| 25 | 43 | 11 | | |
| 26 | 44 | 12 | | |
| 27 | 45 | 13 | | |
| 28 | 44 | 12 | | |
| 29 | 43 | 11 | | |
| 30 | 42 | 10 | | |
| 31 | 42 | 10 | | |
| Jan. 1 | 42 | 10 | | |
| 2 | 42 | 10 | | |
| 3 | 44 | 12 | | |
| 4 | 45 | 13 | | |
| 5 | 44 | 12 | | |
| 6 | 44 | 12 | | |
| 7 | 45 | 13 | | |
| 8 | 44 | 12 | | |
| 9 | 42 | 10 | | |
| 10 | 43 | 11 | | |
| 11 | 45 | 13 | | |
| 12 | 44 | 12 | | |
| 13 | 42 | 10 | 983 | 748 |
| 14 | 43 | 11 | | |
| 15 | 44 | 12 | | |
| 16 | 46 | 14 | | |
| 17 | 46 | 14 | | |
| 18 | 44 | 12 | | |
| 19 | 44 | 12 | | |
| 20 | 43 | 11 | | |
| 21 | 44 | 12 | | |
| 22 | 45 | 13 | | |
| 23 | 46 | 14 | | |
| 24 | 44 | 12 | | |
| 25 | 42 | 10 | | |
| 26 | 42 | 10 | | |
| 27 | 43 | 11 | | |
| 28 | 44 | 12 | | |
| 29 | 46 | 14 | | |
| 30 | 46 | 14 | | |
| 31 | 46 | 14 | | |
| Feb. 1 | 48 | 16 | | |
| 2 | 48 | 16 | | |
| 3 | 47 | 15 | | |
| 4 | 47 | 15 | | 1031 |

x—day planted

## TABLE 2

### Egg Survival of First Main Stream Plant at Woodward's Gate With Average Eggs per Sack and Standard Deviations

| Removal group and date | Original number of eggs | Moldy and/or ruptured | Opaque | Clear unfertilized | Partial develop-ment | Alevins | Number alive | Remarks |
|---|---|---|---|---|---|---|---|---|
| 1A<br>11/ 9/53 | 1,000 | 0 | 0 | 851 | 0 | 0 | 149 | Sacks 1-50 eliminated because of low fertility demonstrated in this sample. |
| $\bar{x}$ | 100 | 0.0 | 0.0 | 85.1 | 0.0 | 0.0 | 14.9 | |
| s.d. | | | | | | | 5.9 | |
| 1B<br>11/ 9/53 | 864 | 1 | 0 | 477 | 0 | 0 | 576 | Eggs from salmon of low fertility eliminated. |
| $\bar{x}$ | 86.4 | 0.1 | 0.0 | 47.7 | 0.0 | 0.0 | 57.6 | |
| s.d. | | | | | | | 11.7 | |
| 2<br>11/20/53 | 893 | 314 | 15 | 348 | 0 | 0 | 344 | Three sacks moved from original positions in stream bed. |
| $\bar{x}$ | 89.3 | 31.4 | 1.5 | 34.8 | 0.0 | 0.0 | 34.4 | |
| s.d. | | | | | | | 28.0 | |
| 3<br>12/ 5/53 | 913 | 281 | 8 | 84 | 36 | 0 | 129 | Seven sacks moved from original positions. |
| $\bar{x}$ | 91.3 | 28.1 | 0.8 | 8.4 | 3.6 | 0.0 | 12.9 | |
| s.d. | | | | | | | 17.4 | |
| 4<br>12/24/53 | 667 | 394 | 6 | 56 | 11 | 0 | 16 | One sack moved from original position. Worms present in one sack. |
| $\bar{x}$ | 95.3 | 56.3 | 0.9 | 8.0 | 1.6 | 0.0 | 2.3 | |
| s.d. | | | | | | | 2.9 | |
| 5<br>1/13/54 | 788 | 137 | 7 | 10 | 12 | 16 | 41 | Six sacks moved from original positions. One apparently normal alevin hatching from moldy shell. Worms in one sack. |
| $\bar{x}$ | 98.5 | 17.1 | 0.9 | 1.2 | 1.5 | 2.0 | 5.1 | |
| s.d. | | | | | | | 5.8 | |

clearing solution of 1 part formalin, 1 part glacial acetic acid, and 14 parts water. Microscopic examination was required to detect early cell stages in those eggs removed shortly after planting. All other eggs showed the embryonic fish very clearly.

Ten of the first 50 sacks, with eggs from the first female, had an egg survival rate of only 14.9 percent (Table 2). The low initial rate left such a small sample for measuring future mortalities that this sample was discarded from the experiment.

Since the eggs from the second female were much smaller, it was easy to distinguish them from the few larger eggs of the first female that were mixed with them in the remaining 50 sacks. The survival rate for the second female's eggs after 3 days was 57.6 percent. Carl (1939), in planting silver salmon (*Oncorhynchus kisutch*) eggs in gravel in a hatchery trough, experienced losses of 46.4 percent. He offers the opinion that high mortalities are the result of extra handling involved in counting the eggs.

Thirteen days later, on November 20, the second group was removed. During this time, the mean temperature of Mill Creek dropped from 55° F. to 44° F. On November 14, during a freshet, the mean stream flow increased to 491 c.f.s.,[2] 7 times the mean recorded at time of planting. This group survived at a rate of 34.4 percent, which is a drop of 23.2 percent from the first removal (Table 2).

A third group was removed on December 5, after 29 days in the gravel. Laboratory examination showed a survival of 12.9 percent. This group had again been exposed to a stream freshet. Mean daily flow for November 24 reached 515 c.f.s.; an increase of over 7 times that recorded when the eggs were planted. Temperature during this time rose from 44° F. to 51° F. and dropped again to 45° F. when the group was removed. The fourth group was collected after 48 days. In the period between the third and fourth removals, flows remained relatively stable. Temperatures fluctuated somewhat, reaching 50° F. and dropping to a low for the season of 42° F. Two and three-tenths percent of the embryos per cartridge were alive. On January 13, the fifth group was removed, after a total of 68 days or 983 heat units. Flows and temperatures remained stable between the fourth and fifth removals. A survival of 5.1 percent was recorded. The eggs were in the process of hatching when removed. Better indicated survival of the last group than the fourth group is a result of sampling error. The daily mean temperatures and stream flows are plotted in Figure 2, along with the various survival rates. Here it can be seen that early freshets were accompanied by rapid declines in survival, while stable flows that followed produced less mortality. A relation between the occurrence of freshets and survival rate was thus suggested.

Freshets the year before (1952-1953) (Gangmark and Broad, 1955) occurred late in the incubation period, in contrast with their early occurrence in the experiment just described. The time of major egg mortality corresponded with the freshets. In 1952-1953 average losses of 4.1 and 7.7 eggs per sack were recorded between the first to second and second to third removals, respectively, when the stream fluctuation

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[2] Water flow records were obtained from the State of California Division of Water Resources. Their stream flow gauge is located one-half mile downstream from Woodward's Gate.

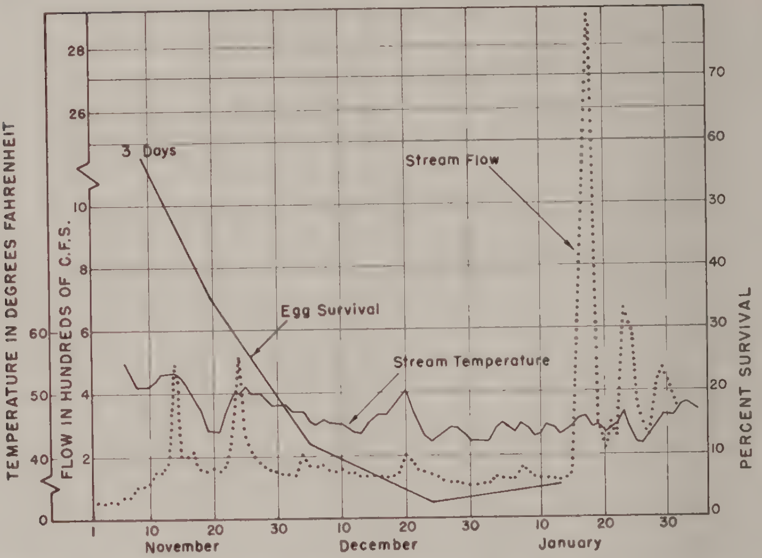

FIGURE 2. The fluctuations in stream flow and temperature of Mill Creek and percentages of survival of egg samples during the first study in 1953-1954.

was moderate. A loss of 18 eggs per sack was experienced between the removal of the third and fourth groups, when the mean flow for one day reached 1,250 c.f.s.

## RESULTS OF THE CONTROLLED FLOW CHANNEL EXPERIMENT

There appeared to be a correlation between occurrence of marked stream fluctuation and survival. Therefore, a controlled flow channel experiment was carried out for purposes of comparison. On November 18, 1953, spawn was taken from two female and three male king salmon. Standard hatchery procedure was followed for obtaining the spawn as before. The eggs were then allowed to water harden for 1½ hours before being placed in the cartridges with some small gravel. Thirty of the containers were destined for Woodward's Gate, as an open channel control, and 31 for the controlled flow area. They were selected alternately for each area and buried in the respective areas simultaneously. Again there were five colors of tags in each group. They were planted by a staggered distribution or by rotating the colored tags. The temperature of the water at this time was colder (44° F.) than during the first plant.

After two days, the first groups were removed. In the main stream, 63.6 percent of the eggs survived the handling operation (Table 3), and in the control channel, 52 percent (Table 4). Between this time and the removal of the second group, flows had increased to a mean of 515 c.f.s. from 152 c.f.s. at planting on November 24. At the end of this period, the cartridges in Mill Creek proper revealed a 37.2 percent survival in the second group, or a drop of 26.4 percent. This is

TABLE 3

Egg Survival of Second Main Stream Plant at Woodward's Gate With Average Eggs per Sack and Standard Deviations

| Removal group and date | Number of eggs found | Moldy and/or ruptured | Opaque | Clear unfertilized | Partial development | Alevins | Number alive | Remarks |
|---|---|---|---|---|---|---|---|---|
| 1 | 496 | 0 | 1 | 177 | 0 | 0 | 318 | One sack removed from stream by a fisherman; eggs destroyed, not counted. |
| 11/20/53 $\bar{x}$ | 99.2 | 0.0 | 0.2 | 35.4 | 0.0 | 0.0 | 63.6 | |
| s.d. | | | | | | | 11.4 | |
| 2 | 591 | 137 | 0 | 231 | 0 | 0 | 223 | Two sacks moved from original position. |
| 12/ 5/53 $\bar{x}$ | 98.5 | 22.8 | 0.0 | 38.5 | 0.0 | 0.0 | 37.2 | |
| s.d. | | | | | | | 26.3 | |
| 3 | 589 | 291 | 8 | 69 | 61 | 0 | 160 | |
| 12/24/53 $\bar{x}$ | 98.2 | 48.5 | 1.3 | 11.5 | 10.2 | 0.0 | 26.7 | |
| s.d. | | | | | | | 9.6 | |
| 4 | 379 | 85 | 9 | 120 | 29 | 0 | 136 | |
| 1/13/54 $\bar{x}$ | 63.2 | 14.2 | 1.5 | 20.0 | 4.8 | 0.0 | 22.7 | |
| s.d. | | | | | | | 14.1 | |
| 5 | | | | | | | | No eggs recovered. |

the same time interval as between the second and third groups in the first experiment, in which a 21.5 percent loss occurred. The rates of loss in the two experiments for the same period were somewhat similar. In the controlled flow area 53.8 percent of the eggs were alive after this period, indicating that sampling error was responsible for a lower survival of the first sample. However, there is a clear difference in survival rates between the two areas. Eggs in the main stream suffered a substantial loss as compared to an apparently good survival rate in the control area (Figure 3).

In the period that followed until the third group was removed, when the salmon embryos were 36 days old, the temperatures and stream flows remained relatively stable. The survival rates in the two areas were quite similar. The main stream survival was 26.7 percent, a drop of 10.5 percent since the previous sampling. Embryos in the controlled flow area survived at a rate of 44.4 percent, a drop of 9.4 percent.

At the end of 56 days, the eggs were sampled for the fourth time in the two areas. In the main stream, 22.7 percent were alive, an average of 4 less per cartridge than in the previous sample. In the control area, 37.8 percent were alive, for an average of 6.6 embryos per container less than in the previous group. Again the temperature and stream flow factors remained stable for this period.

After the fourth group had been removed, stream flow suddenly rose to a high for the year. The water gauge at Mill Creek recorded a mean of 2,740 c.f.s. for the 17th of January. This represented a flow of 18 times the mean recorded on the day the eggs were planted. It will be recalled that the experimental area at Woodward's Gate was specifically chosen for its ability to carry large volumes of water. How-

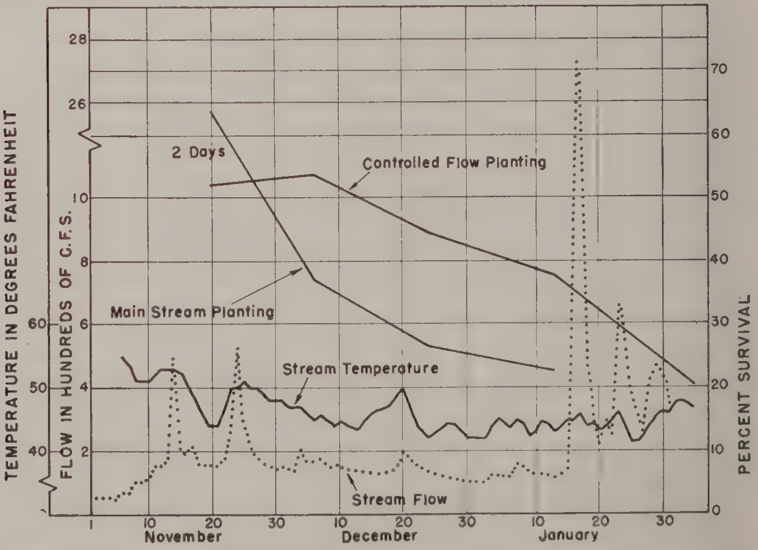

FIGURE 3. The fluctuations in stream flow and temperature of Mill Creek and percentages of survival of egg samples during the controlled flow and second main stream study in 1953-1954.

TABLE 4

Egg Survival of Controlled Flow Area Plant With Average Eggs per Sack and Standard Deviations

| Removal group and date | Number of eggs found | Moldy and/or ruptured | Opaque | Clear unfertilized | Partial develp-ment | Alevins | Number alive | Remarks |
|---|---|---|---|---|---|---|---|---|
| 1 | 599 | 1 | 0 | 286 | 0 | 0 | 312 | |
| 11/20/53 | | | | | | | | |
| $\bar{x}$ | 99.8 | 0.2 | 0.0 | 47.7 | 0.0 | 0.0 | 52.0 | |
| s.d. | | | | | | | 18.3 | |
| 2 | 556 | 181 | 0 | 52 | 0 | 0 | 323 | |
| 12/ 5/53 | | | | | | | | |
| $\bar{x}$ | 92.7 | 30.2 | 0.0 | 8.7 | 0.0 | 0.0 | 53.8 | |
| s.d. | | | | | | | 7.5 | |
| 3 | 673 | 301 | 16 | 20 | 25 | 0 | 311 | |
| 12/24/53 | | | | | | | | |
| $\bar{x}$ | 96.1 | 43.0 | 2.3 | 2.9 | 3.6 | 0.0 | 44.4 | |
| s.d. | | | | | | | 14.8 | |
| 4 | 268 | 30 | 0 | 0 | 11 | 0 | 227 | Six eggs with mold on shells classified as alive. |
| 1/13/54 | | | | | | | | |
| $\bar{x}$ | 44.7 | 5.0 | 0.0 | 0.0 | 1.8 | 0.0 | 37.8 | |
| s.d. | | | | | | | 14.0 | |
| 5 | 240 | 52 | 17 | 7 | 41 | 45 | 123 | Alevin count included in "alive." |
| 2/ 4/54 | | | | | | | | |
| $\bar{x}$ | 40.0 | 8.7 | 2.8 | 1.2 | 6.8 | 7.5 | 20.5 | |
| s.d. | | | | | | | 5.5 | |

ever, the force of the current was sufficient to change the profile of the riffle and to dislodge the salmon embryos in the gravel.

No evidence remained of the cartridges which were to be removed on February 4, after 78 days. The final tally was 20.5 percent alive for the controlled flow area, a decline of 17.3 salmon embryos per cartridge since the last removal. The stream temperature and flow records are shown in Figure 3 in connection with survival rates in the two areas.

## RESULTS OF THE JANUARY 17, 1954, FRESHET

The high velocity flows which occurred January 17 thoroughly flushed and changed the riffle area at Woodward's Gate. Stones of varying size which had been painted white, numbered in black, and placed individually on each redd were no longer in position over the nests. Thirteen of the stones were found downstream at distances varying from 8 feet for a 9-pound stone to 525 feet for a 1.6-pound stone. The distances were measured from the downstream end of the planting area because most of the numbers had been worn from the stones.

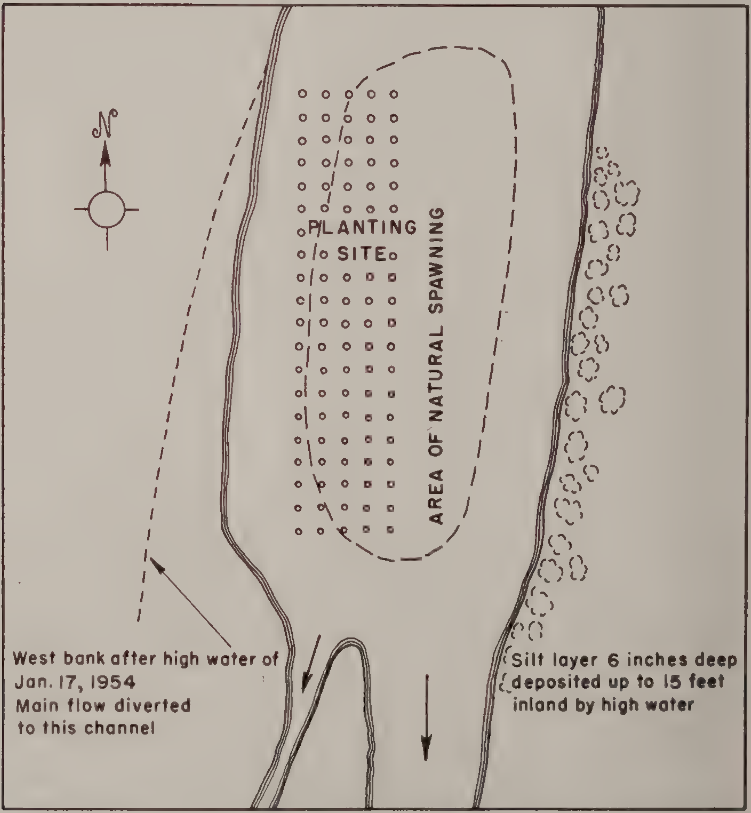
N
PLANTING SITE
AREA OF NATURAL SPAWNING
West bank after high water of Jan. 17, 1954 Main flow diverted to this channel
Silt layer 6 inches deep deposited up to 15 feet inland by high water

FIGURE 4. Sketch of Mill Creek planting site at Woodward's Gate.

The profile of the riffle at Woodward's Gate was changed markedly. A channel 18 inches deep was gouged through the area where 30 pairs of king salmon had spawned. The main stream was diverted from the east to the west side of a little island shown in Figure 4 and 6 inches of silt was left on the east bank up to 15 feet from the water's edge. A check was made to determine to what extent the natural spawn was affected. Nests were explored with a hoe, and a hand net was used to catch any embryos which might be dislodged. From other samples of eggs, we estimated that the majority of the natural spawn would be in the early alevin stage, when the young fish require gravel to protect them from the pull of the current and from predators. Persistent digging produced no eyed eggs or alevins.

In an attempt to evaluate roughly the extent of main stream damage, other areas were excavated in a similar manner. Below Ward Dam the stream is divided into three channels. In the two smaller channels, each of which contains less than 25 percent of the total flow of Mill Creek, eyed eggs and alevins were discovered in the gravel. Such small channels appear to be of considerable value in maintaining salmon runs, since the effect of flooding is less violent.

Evidence of loss of spawn was found at the mouth of Mill Creek, where water from the Sacramento River temporarily backed into Mill Creek. Several thousand dead eyed eggs were seen floating on the surface after the January 17th freshet. Also, 51 eyed eggs were found in the stomach of a 12-inch rainbow trout caught by a local sportsman after the flood.

## DISCUSSION

Of the various factors that might have affected egg survival in our experiments, flooding is the most apparent. This influence has been so overwhelming that it has overshadowed other factors. More data are always desirable, but the effect of freshets in both the 1952-53 and 1953-54 work cannot be overlooked. This conclusion is based on the following: (1) the close correlation existing between egg losses and freshets in which stream flow increased by seven or more times; (2) the opposite survival trends for the two years in which heavy losses and extreme flows occurred late in 1952-53, compared with early losses accompanying freshets in 1953-54; and (3) the heavy egg losses experienced during an early period in the main stream in two experimental plants, in comparison with little or no egg loss during the same period in the control flow channel in 1953-54.

The influence of freshets should not be confused with that of substantial stream flows. Adequate flows are especially important in the Sacramento Valley in California. First of all, the upstream migrants must have sufficient water to swim in and there must be enough water to maintain suitable temperatures and oxygen supply. Second, stream flows must provide adequate area for adults to spawn without crowding. Third, there must be sufficient water flow throughout the incubation period to keep the eggs alive. Fourth, strong flows during downstream migration increase turbidity and protection from predators, lessen the chance of the small fish being swept into irrigation intakes, dilute pollutants, and stabilize temperatures and oxygen conditions in the streams.

As in the case of the analyses made on the data of 1952-53 (Gangmark and Broad, 1955), no significant difference in survival related to position of the cartridges in the stream was found. The analyses covering the two seasons indicate that with the exception of the flow control area, position of the cartridges in the stream has not significantly affected survival in these experiments.

## ACKNOWLEDGMENTS

Grateful acknowledgment is extended to Robert Azevedo of the Branch of Game-Fish and Hatcheries and to several members of the California Department of Fish and Game, for their assistance in conducting the experiments described herein. We wish also to express sincere thanks to Scott Bair, Hydraulic Engineer, Fish and Wildlife Service Regional Office in Portland, Oregon, for surveying and providing plans for construction of the flow control dam which was built.

## SUMMARY

Because of the correlation of king salmon egg mortality between the stages of fertilization and hatching in Mill Creek, California, with stream freshets in 1952-53, a controlled flow area was established to further investigate the problem in 1953-54.

The technique for conducting the experiment involved enclosing fertilized eggs with gravel in plastic screen sacks and burying them in the streambed. The sacks were removed at five different stages and various influences that may have affected survival were noted.

In one experiment involving 50 such sacks planted at Woodward's Gate, early losses were noted in the first two periods, in each of which a freshet occurred. The mortality rate was comparatively light during the last two periods, when stream flows remained stable.

Early mortalities during 1953-54 accompanied early freshets. In 1952-53 mortalities were at first small, while stream flows were stable. Mortalities increased with the occurrence of the first freshet, with complete loss following a subsequent flood.

A second experiment in 1953-54 involved placing eggs in the Woodward's Gate section and planting a similar group in the controlled flow channel. Main stream losses were heavy and in sharp contrast to a good survival rate in the control channel.

In the two periods which followed this, stream flows remained moderate and survival rates were comparable in the creek and controlled flow area until January 17, 1954, when a freshet occurred.

Results of this freshet on the Woodward's Gate area were: (1) complete erosion of the streambed; (2) loss of experimental cartridges and natural spawn from the riffle; and (3) a change of the channel and a heavy deposit of silt on the banks. Eggs in the controlled flow area suffered mortalities from silt deposition only. After the freshet only areas in which the rate of flow was reduced by the stream dividing and subdividing produced eggs and alevins.

Further indication of spawn losses after the flood were numerous floating eyed eggs which were seen at the mouth of Mill Creek. A rainbow trout caught after the freshet was found to contain many king salmon eggs that had been eroded from the gravel.

Severe freshets or violent stream fluctuations should be distinguished from substantial stream flows, which are beneficial to virtually all phases of the freshwater life history of salmonids.

An analysis of variance revealed that position in the stream had not significantly affected survival in these experiments.

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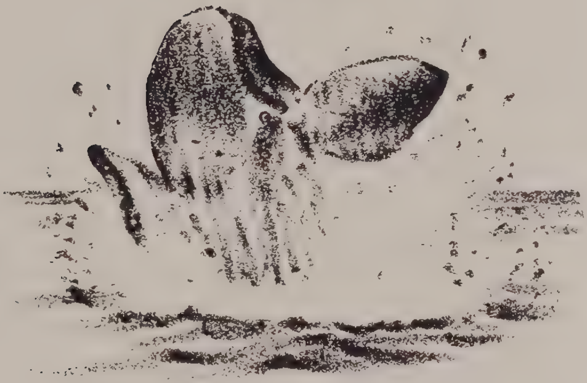
# RARE RIGHT WHALE VISITS CALIFORNIA

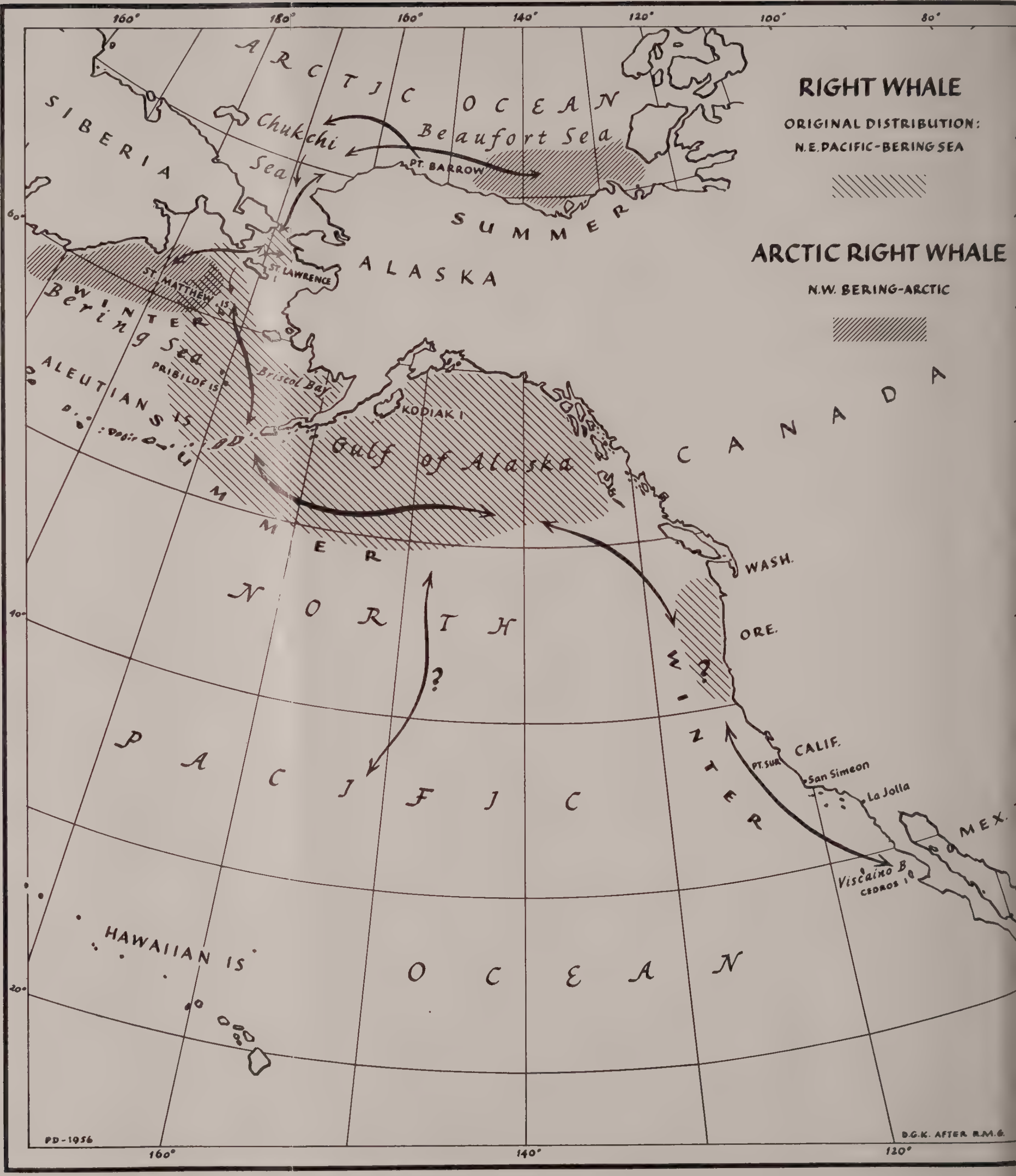
*Raymond M. Gilmore*

Reprinted from PACIFIC DISCOVERY (Vol. IX, No. 4, July-August 1956, pp. 20-25), a bi-monthly publication of the California Academy of Sciences, San Francisco.

# RIGHT



# WHALE


RIGHT WHALE
ORIGINAL DISTRIBUTION:
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ARCTIC RIGHT WHALE
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ARCTIC OCEAN
Chukchi Sea
Beaufort Sea
PT. BARROW
SIBERIA
SUMMER
ALASKA
ST. LAWRENCE I
ST. MATTHEW IS
WINTER
Bering Sea
PRIBILOF IS
Bristol Bay
ALEUTIANS IS
KODIAK I
Gulf of Alaska
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# are RIGHT WHALE Visits California

WHALES MAKE NEWS, *and rare ones make special news. Such a rare whale is the right whale, also called the black whale, or the bowhead. The special news, in this case, is its visit to La Jolla, southern California, in 1955.**

THE RIGHT WHALE was formerly one of the commonest and most important of all the whales of the world. It, with the sperm whale, was the hub of the old hand-whaling industry. Like the sperm whale, the right whale swam slowly when at the surface and allowed close approach by rowed or sailed whaleboats. It floated when dead, thus permitting recovery.

It was world wide in distribution, and in the northeastern Pacific a large group concentrated in the summer in the Gulf of Alaska. These were called the "Northwest Whale" by the early American hand whalers, and were almost killed off in the latter half of the 19th century before their winter range had been well defined.

Captain Charles M. Scammon in his classic account of the whales of northwestern North America, published in 1874, mentioned that right whales "were found on the coast of Oregon, and occasionally in large numbers." Presumably this was in winter. Scammon made no mention of any right whales taken off the Hawaiian Islands, but he did state that some stragglers were captured off the California coast, down even as far as Sebastian Vizcaíno Bay in mid-Baja California where ends one of the last eddies of the vast north Pacific current of cold water with its associated species. Nor did C. H. Townsend, in his monumental analysis of the captures of whales by the early Yankee whalers, show the right whale as occurring off California or the Hawaiian Islands. (See references at end.)

We can postulate, accordingly, that the normal *winter* range of the Northwest right whale was off the coast of Oregon and possibly northern California, and that occurrences off southern California and northern Baja California were by stragglers only. This main winter range was never exploited by the whalers, probably because of inclement weather during that season, and because the right whale might have been more concentrated, and certainly was easier to get in the summer on the Kodiak grounds of the Gulf of Alaska and the adjacent southeastern Bering Sea.

### Many whales, few of right kind

The coast of California is rich in whales, winter and summer, and has been heavily whaled since the 1850's, with a lull between 1890 and 1920. From 1850 to 1890 there were at least 15 shore stations from San Francisco to San Diego. Most of these stations operated in the winter season for gray whales; but this season, incidentally, is also the "right" time of year for the "right whale," although few were taken.

Fragmentary catch statistics from these early days, tabulated by David Starr Jordan in 1887, by C. H. Townsend in 1887, and by Edwin C. Starks in 1922, showed that at Point Sur in the winter of 1878–1879 one right whale was taken with three grays and one humpback (Jordan); at San Simeon in the winter of 1884–85 three right whales were killed along with 15 grays (Townsend, Starks); and that at San Diego a right whale was occasionally taken from 1850 to 1870, which was as long as the gray whales lasted (Jordan). More records will certainly be revealed by examination of old newspaper files of the whaling towns, but it is doubtful that the right whale will be found to have occurred commonly in the catches.

With the upsurge of whaling by steam catcher boats and steam reduction plants, together with the powder harpoon-cannon, in the 1920's and 1930's, some 3,000 whales were killed on the California coast; but among them was only one right whale, off the Farallon Islands in April, 1924. Most of these 20th century operations were in summer, however, the wrong season for the right whales, so most were surely missed. Even in this recent period, our statistics of the catches from the floating factories are not complete, and investigations may reveal other right whales.

### Extinct in California? . . .

Thus, for all practical purposes, the northeast Pacific right whale was gone from the coast of California. In 1933, Joseph Grinnell in his *Review of the Recent Mammal Fauna of California,** said of the right whale: "Fairly common, formerly, along coast of California; but has not been reported since about 1885 when found near San Simeon."

And Lloyd G. Ingles, author of the recent *Mammals of California and its Coastal Waters,*† concluded that the right whale is "now very rare, perhaps no longer extant in California." Neither author knew of the 1924 capture.

### . . . Then in 1955 . .

And so we come down to March 31, 1955, when the next right whale was definitely seen off the coast of California.

On this day a whale appeared off the Scripps Institution of Oceanography in La Jolla. It came from the north, unlikely for any whale at this season when the grays were migrating in the opposite direction. It almost struck the piles of the pier, veered off seaward and turned north. Then, immediately reversing its course, it continued southward again.

All this was seen from the windows of the Scripps

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*Univ. Calif. Pub. Zool., 40.

†Stanford University Press, 1954.

Institution of Oceanography laboratories and classrooms, and the whale was quickly noted as different from a migrating gray, both in behavior and appearance.

With its turn south, I took up station at the whale watch atop Ritter Hall, built for gray-whale counting and for just such observations as this. Here I watched the whale through 18-power binoculars. It continued south close to shore but was soon blocked by Point La Jolla, a mile away. At this place, next to the Cove and cliffs, with many strollers and bathers watching, the whale for 40 minutes rolled, turned, fluked, loblolled with flipper out, stretched its back in a sinuous curve, and apparently examined the shore time and time again, with head partly out of water — yes, distinctly looked over the surrounding water and nearby land, as if trapped or confused by the curving shore line, and perhaps also by the deep "La Jolla" submarine canyon, over which it just passed.

All this maneuvering showed unmistakably that it was a right whale: the massive broad body; the smooth back without fin or ridge; the black, unblotched color; the wide flukes with sharp wing-tips; and especially the widely divergent double spout; the scabrous bump on the tip of the down-curved snout; and the huge lower lips which projected upward and partially overlapped the narrow, down-curved upper jaw.

Five minutes later a small boat swung out over the end of the Scripps pier and into the water. Into it jumped Don Lear, Eddie Arabelo, and myself, with cameras and outboard motor — the chase was on.

Speeding to the Cove, we arrived to find the whale gone, and so we immediately turned out to the end of the La Jolla kelp beds, a mile west. There we soon saw the double spout and the huge, smooth, black back of the whale. Then for 20 minutes we hung on its flank and flukes, 50 to 200 feet away, at a speed of five to six knots, which kept us close and gave us momentum to turn quickly had the whale done the same. We estimated its length to be 40 feet.

As if in response to this annoyance of motor and boat, the right whale breached eight times almost clear of the water, once five times in succession, and then three. At each jump, the whale's ponderous bulk rose slowly and majestically out of the water, belly uppermost with flippers outstretched like barn doors, and fell back with a tremendous shower of spray and white water. It was an extremely impressive show of size and power — I think the whale intended it to be.

That such jumping or breaching was a response to our invasion of the water, or to disturbance by our boat, is supported by a series of five jumps by this same whale a short time previously near the Cove when it was approached by a passing lobster-skiff. The gray whale is also a great breacher and sometimes can be stimulated to do so by approach of a small boat with motor running. At least this is true in these southern waters in winter during the gray whale's mating and calving season.

Finally the chase ended as the whale took off to the southwest, without visibly surfacing, as if deliberately to throw us off the track. Such evasive or furtive swimming tactics are common to whales when frightened. At such times they expose only the nostrils to breathe, without showing either the head or back. The breath is also released so slowly that it does not condense into a visible spout. Consequently, unless one's eye is right on the target, the whale can easily escape unnoticed.

### He kept his big mouth shut!

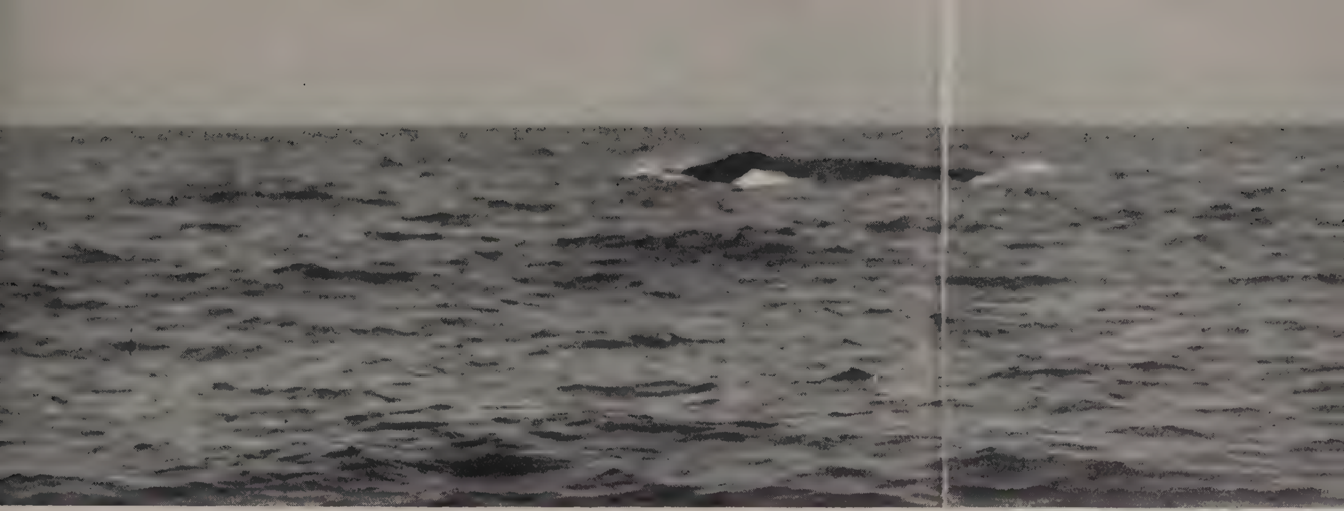
It was noted that the mouth of this right whale was closed at all times, though the figures in most books, reproduced year after year, edition after edition, show the mouth partly open. Such figures seem to have been drawn from dead and stranded individuals, in which the tongue was swollen, forcing the mouth open. Or perhaps the open mouth of the pictures is meant to show a feeding posture, which is certainly more like the figures.

Ordinarily, however, the right whale has the mouth closed, and this brings into view the high, prominent, thick lower lips which stick up on each side to overlap the down-curved, narrow upper jaw. This is such an excellent field character that, on the schematic drawing here appended, I have shown the mouth closed and lower lip high as we actually saw it. Recent photos of right whales at the surface on the east coast of North America also show a closed mouth and conspicuous lower lip.

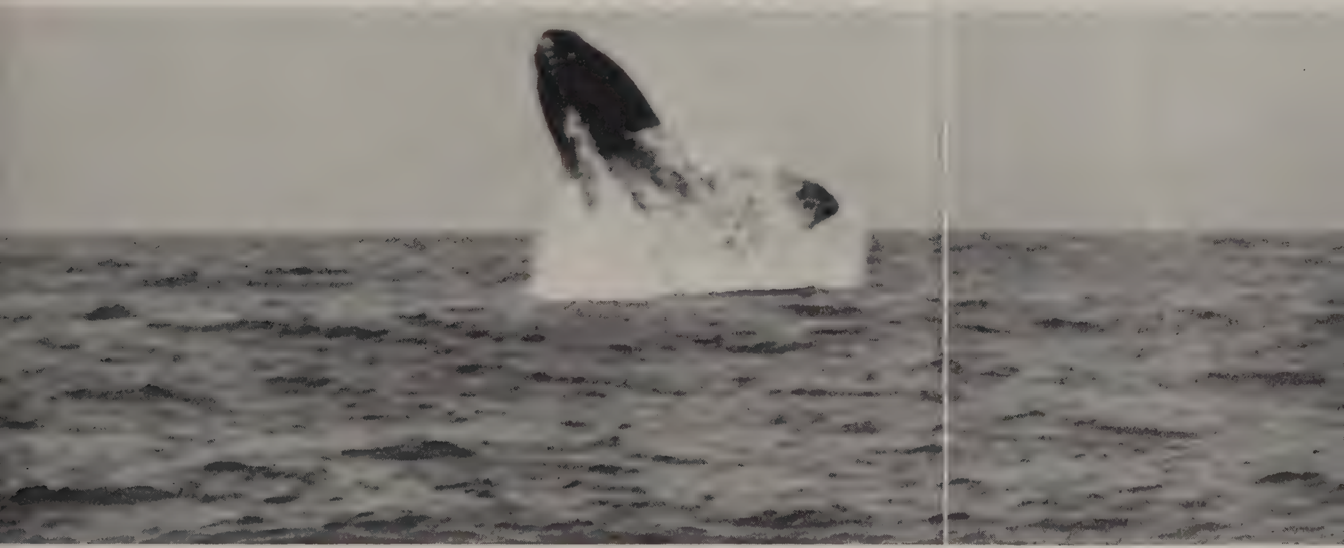
### . . . A comeback?

This particular right whale was a very welcome sight indeed, and others perhaps were in the vicinity of San Diego in early 1955. On 14 January two volunteer gray whale-watchers, atop the lookout at Ritter Hall on the Scripps campus, saw four whales at a distance of 3 to 4 miles going southeastward. These whales were described as showing a distinct double spout, a smooth black back, and pointed, broad flukes. They were logged as "gray whales," a species which was then migrating south in numbers, and which also sometimes shows a faint double spout at close range from directly fore or aft, and also has no fin on the back. The double condition of the observed spouts in this case was too great for the gray whale, however, and the back and tail-stock were without the slight but easily visible ridge and sharp knuckles, and no clear patches of light, discolored areas were present.

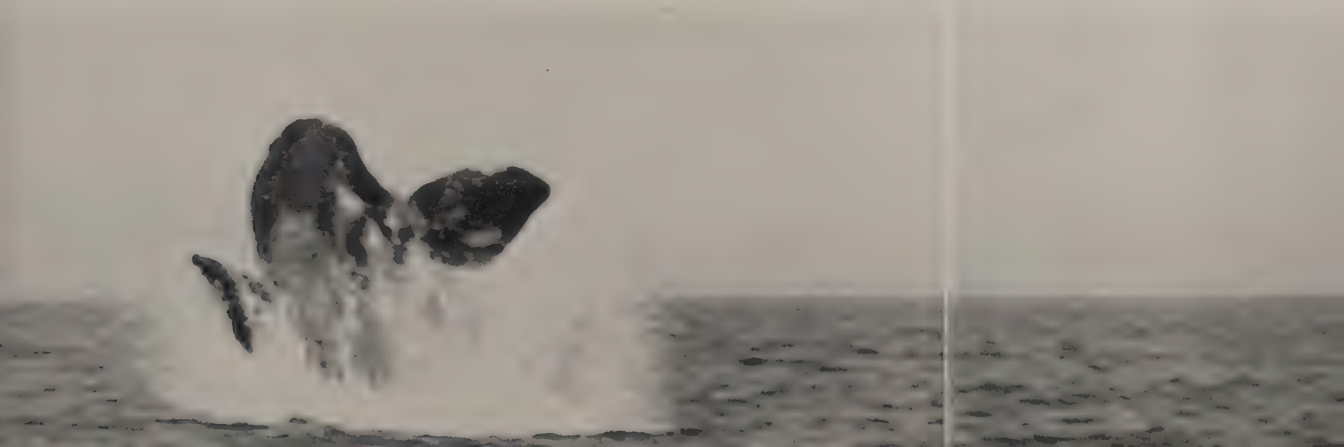
In May 1951 the Western Whaling Corporation of Coal Harbour, Vancouver Island, shot a right whale by mistake, thereby adding to our knowledge of the distribution of the species farther north.

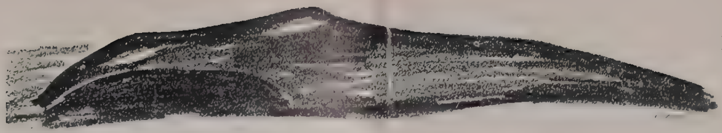


Pacific right whale at La Jolla, California, March 31, 1955. (ABOVE) Body rises to blow at close range. The peaked top of the head represents muscles bunched for the opened blowholes and its highness is typical of the bowed head of the right whale.

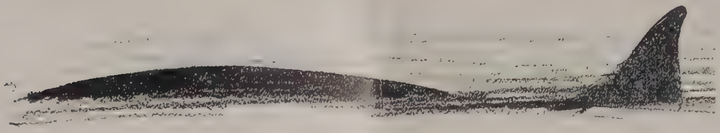


(CENTER AND BOTTOM) The whale breaches out of the water with back down, belly up, and flippers outspread, apparently in response to the challenge of a small boat with outboard motor. (U. S. Fish and Wildlife Service photographs by Dr. Raymond M. Gilmore)

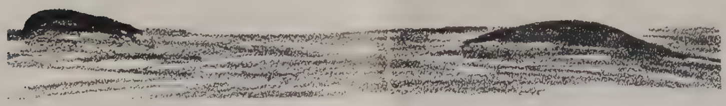




Field sketch of the right whale's head peak.



(ABOVE) Whale lolling on its side. (BELOW) Curving the back with snout and tail stock out of water.



Though the right whale will never become common off southern California because this area is outside the normal range, it is reasonable to look forward to a repopulation of the Oregon coast and perhaps that of northern California, with stragglers from year to year farther south.

### Around the world, a whaler's whale

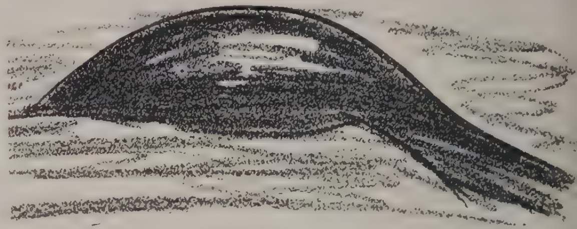
To return to the world-wide distribution of the right whale and its historical importance: There was a large European group which wintered in the Bay of Biscay and summered around the Shetlands, Orkneys, and Norway. In Biscay Bay it was the object of the *first whaling industry,* that of the Basques, starting during the 11th century, or possibly even the 10th. At this time the techniques of hand-harpooning and lance-killing were perfected, though for centuries or millenia, similar techniques had been used by primitive man such as the Eskimo. However, the Basques also held onto their whales with the harpoon line attached to the small boat, a distinct improvement over the technique of floats on the line as used by primitive man.

The products of the right whale were the great quantities of clear and limpid oil, rendered ashore. This oil was used for illumination and cooking. The long, fine-fibered and silky-fringed baleen or whalebone was also prized, being used to stiffen certain undergarments the ladies wore even then.

Off the coast of eastern North America, in the western North Atlantic, another group wintered from Long Island to Florida, and summered on the Grand Banks of Newfoundland to Iceland. Our Yankee forefathers cut their whaling teeth on this group, pursuing it relentlessly from 1650 to 1750 with the same techniques of the Basques. The last hand shore-whaling for the American right whale was on Long Island in 1918.

With the early decline of this local group, pelagic whaling was adopted by the Yankee who took to the oceans of the world in search of the right whale and the sperm whale. These Yankee forefathers of ours would stay at sea for one, two, and even three years, trying-out the whale oil aboard ship. The pelagic techniques had been developed also by the Basques, and had been perfected by the English in their pursuit of the Arctic right whale.

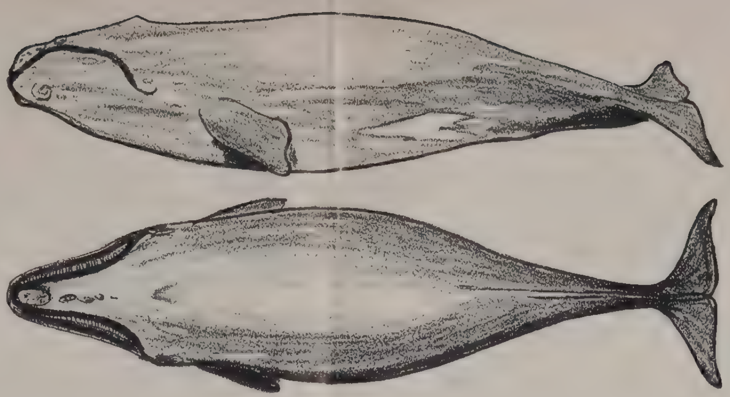
The pelagic whaler first worked the oceanic summer feeding grounds of the south Atlantic: the Brazil and False banks and Falkland Grounds off southeastern South America; then across the ocean through the Pigeon Ground, the Tristan Ground, South African Ground, and to the Woolwich Bay Ground on the southwest coast of Africa. From here the whalers penetrated both the Indian and Pacific oceans, whaling the grounds of Delagoa Bay, off southeastern Africa; the Crozet, Desolation, and Indian Ocean grounds near the subantarctic; the Coast of Chile Ground; the Kermadec Grounds northeast of New Zealand; the New Zealand Grounds; the Coast of New Holland Ground off southwestern Australia; Sea of Japan, or Korean, Ground; the Okhotsk and Kamchatka grounds; and finally the Kodiak Grounds — which brings us back to the beginning of our story.



Base of tail rolling high when sounding — flukes not thrown on this occasion.

Some of these grounds are oceanic, other insular-oceanic, others coastal; it all depended on the season, the activity of the whale, whether breeding or feeding, and the fertility of the water mass if it was a feeding ground.

By a freak of economic forces the right whale and its close cousin, the Arctic right whale, were almost completely exterminated, which would have been the first such case for a pelagic mammal in history. Before the invention of springy, light, and stainless "featherbone" steel about 1910 that replaced the whalebone slips and fibers in foundation garments and heavy silks, the demand for the natural product rose to the fantastic heights of $5.00 to $7.00 a pound. This raised the value of a single adult right whale, yielding perhaps 1,500 pounds of "bone," to the point where but one or two whales would pay for a voyage. As a result, almost the last of them were hunted down relentlessly. Ordinary economic forces, which would have made it unprofitable to go to sea for such a small catch, were in this case ineffective. "Featherbone" came almost too late. Now, thanks to the world-



**Schematic drawing of right whale based on photos and field observations. (Raymond M. Gilmore)**

wide protection afforded by international law, right whales are increasing, though their numbers are still small.

There is a theory that reduction in size of a population of wild animals, or of the species itself, to a certain critical, low level, but still considerably above that of the last male and female, causes reproduction to cease or to slow up so that the group in question becomes extinct.

This level may be called the "threshold of populational survival," or the "threshold of extermination." It has been called the "minimum population." Its position is obviously difficult or impossible to determine in nature — until it is too late to save the species. Nevertheless, the populations of the right whale, by the freak of economic forces above described, demonstrated that this level can be extremely low, and that perhaps only a few score individuals in this case saw the species through the crisis of near extinction. Male managed to meet female, or group to remain in contact, despite the great expanse of ocean which they made their home.

Recovery of the right whale to former levels of abundance will take many years. The right whale is still scarce, despite the long time elapsed since whaling for the species ceased, and the shorter time of complete protection.

## Whales as oceanographers

The right whales were attracted to their summer grounds along the coast or in mid-ocean by rich pastures of concentrated small animals, or zoöplankton, such as pelagic shrimp and copepods. These tiny creatures were themselves concentrated there because of fertile water, enriched by currents which bring high-nutrient subsurface waters to the surface layers. Certain coastal spots where upwelling occurs also have rich waters. The Kodiak ground is the site of the slow but enormous Alaska or Kodiak Gyre; the southeastern Bering Sea of the small Bristol Gyre — both summer feeding grounds of the "Northwest Right Whale." In the winter season, however, when whales are breeding, feeding appears to play only a secondary role in choice of locality.

It is to these fertile areas of the ocean that man is now turning for his marine resources, particularly food. And, whales in summer in large numbers may indicate just such areas. Knowledge of the species of whales and of their favorite food enables the oceanographer to gain a quick understanding of the nature and location of concentrations of organisms in what appears to be, to the untrained eye, huge and apparently uniform areas of ocean. The early whalers were among the first true oceanographers. Whales are even better oceanographers than whalers.

Oceanography has recently developed mainly along the lines of indirect observation, by complex instrumentation, of things hidden from our sight. With the resumed interest in whales, these very obvious inhabitants of the ocean can once again take an important role in the science of the sea.

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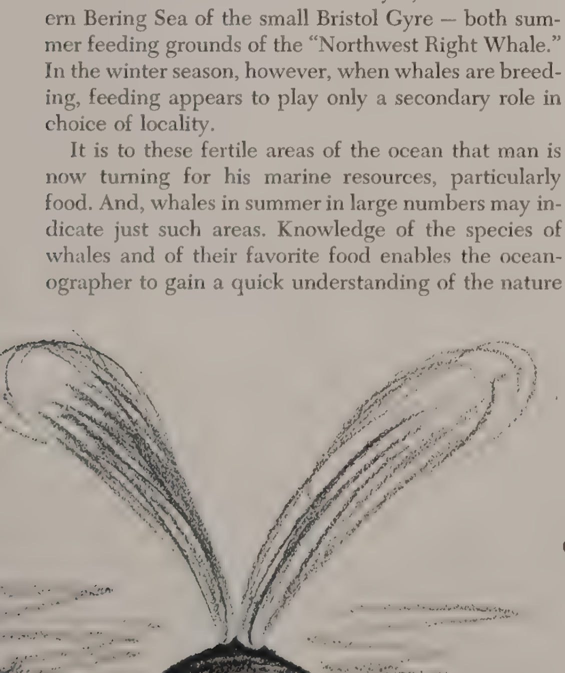
*For those who wish to consult the references mentioned in the text or to read further about the right whale and whaling along the coast of California, the following will be helpful.*

JORDAN, DAVID STARR. 1887. "Coast of California." Pages 52-56, in "The Whale Fishery," by A. Howard Clark, which is part XV of *The Fishery and Fishery Industries of the United States,* edited by George Brown Goode, itself a section 5, volume 2, of the *History and Methods of the Fisheries,* Washington, Government Printing Office. This was a monumental series of 8 volumes of books, and had many contributors. The quoted article by David Starr Jordan, who was one of the great students of fish in our country and a long-time former president of Stanford University, dealt with open boat whaling on the coast of California. The set of books is still very valuable, especially from a historical standpoint, and is becoming scarce.

SCAMMON, CHARLES MORRILL. 1874. *The marine mammals of the northwestern coast of North America described and illustrated: together with an account of the American whale-fishery.* Published by John H. Carmany and Co., San Francisco, and G. P. Putnam's Sons, New York, pages 319 plus v, plates 37. Scammon was undoubtedly the most literary and scientifically inclined of the early whaling captains, and his observations were charmingly set down in this classic book. Though some of the data are no longer authentic or complete, the historical aspects are still the best ever written. This is a rare book; and, with the recent increase of interest in whales, especially the California gray whale along the coast of California, the price has become exhorbitant.

STARKS, EDWIN C. 1922. "A history of California shore whaling." California Fish and Game Commission, Sacramento, Fish Bulletin 6, pages 38, figures 22. Starks presented a valuable summary of the shore whaling stations and their catch in the early days. The fragmentary na-



**Characteristic V-spout of right whale. (Sketches by Raymond M. Gilmore)**

ture of these data was partly unavoidable; we scarcely know more today. A perusal of old newspaper files and personal letters of pioneer California whalers by anyone today will certainly reveal details which will fill in the gaps.

Townsend, Charles Haskins. 1887. "Present condition of the California gray whale fishery." Bulletin of the U. S. Fisheries Commission for 1886, volume 6, pages 346-350, plates 6 and 7. Townsend was one of the giants of the early investigations on marine subjects, and in 1886 made a visit to California to see shore whaling of the California gray whale at first hand. He spent most of his time at the station at San Simeon, where Captain Packard, the owner, gave him much information, that included the capture of a right whale as mentioned in the present paper.

Townsend, Charles Haskins. 1935. The distribution of certain whales as shown by logbook records of American whale ships. *Zoologica,* Scientific Contributions of the New York Zoological Society, volume 29, number 1, pages 50, figures 2, plates 4 (maps). Published on 3 April. When almost 80 years of age, Charles Townsend published, with the help of Mr. Arthur C. Watson, then of the Whaling Museum of New Bedford, the mapped distribution, by month, of the captures by the Yankee whaling ships (as shown in their logbooks) of 53,879 whales, of which 2,118 were right whales. This was a magnificent study and, when properly used, with a realization that only four species were represented and that other grounds have subsequently been or were previously exploited, can be fitted accurately into our present knowledge of whale distribution.

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# Some Unusual Fishes From the Central Pacific

JOSEPH E. KING and ISAAC I. IKEHARA[1]

THE PURPOSE OF THIS ARTICLE is to call the attention of ichthyologists to certain rare or uncommon fishes recently collected in the central Pacific by staff members of the Pacific Oceanic Fishery Investigations at Honolulu, and others. These fishes have been deposited with the U. S. National Museum. The photographs included here were taken by E. D. Stroup; Figure 4 was prepared by Tamotsu Nakata.

## SQUALIDAE

The first two species, here placed in the Squalidae following Hubbs and McHugh (1951), were formerly placed in the Dalatiidae, e.g. Bigelow and Schroeder (1948).

### 1. **Euprotomicrus bispinatus** (Quoy and Gaimard)

One female specimen (Fig. 1) (USNM No. 164176), 161 mm. in total length. Collected March 3, 1948, about 340 miles WNW of Johnston Island at 18°24′N., 175°12′W. in water about 1,000 fathoms deep. Attracted to a light and dip-netted at surface. Collected by M. B. Schaefer aboard M/V "Oregon" operated by Pacific Exploration Company.

*Description:* No anal fin; 2 dorsal fins without spines; snout blunt and broadly rounded; 5 gill openings, all anterior to pectorals; body nearly cylindrical; eyes lateral without nictitating membrane; a large spiracle present; mouth transverse with a groove extending posteriorly from each end; teeth with only 1 cusp and not serrated; upper teeth narrowly triangular and needle-pointed, lower teeth broader, compressed, and bent outwards; caudal axis scarcely upturned; no subterminal notch evident; end of base of first dorsal fin well in advance of the pelvic insertion; base of second dorsal fin very much longer than that of the first; denticles quadrate, with a central pit; first dorsal fin very much nearer pelvic base than pectoral base; body uniform brownish black (in alcohol after fixation in formalin), outer edges of fins clear. Measurements of the specimen and proportional measurements as per cent of total length are given in Table 1.

Hubbs and McHugh (1951) report the capture of a female *E. bispinatus*, 233 mm. total length, about 500 miles off the California coast. They give a thorough description of the specimen and state that it is ". . . the only extant example of the genus known from the Pacific Ocean and, so far as we know, the only one in an American museum." An earlier specimen collected in the open Pacific "between Honolulu and San Francisco, but nearer to the former" (Eigenmann, 1891) was destroyed in the San Francisco fire, according to Hubbs and McHugh. Since we can find no record of any capture of this species since

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TABLE 1

BODY MEASUREMENTS AND PROPORTIONAL MEASUREMENTS OF *Euprotomicrus bispinatus* AND *Isistius brasiliensis* (Body Measurements in Millimeters, Proportional Measurements [bracketed] in Percentage of Total Length)

| | *E. bispinatus** | *I. brasiliensis*† |
|---|---|---|
| Trunk at origin of pectoral: breadth | 19.0 (11.8) | 21.0 (11.1) |
| height | 17.0 (10.6) | 19.5 (10.3) |
| Snout length in front of: outer nostrils | 3.0 ( 1.9) | 1.5 ( 0.8) |
| mouth | 17.0 (10.6) | 13.0 ( 6.9) |
| Eye: horizontal diameter | 6.0 ( 3.7) | 8.0 ( 4.2) |
| Mouth: breadth | 11.0 ( 6.8) | 10.5 ( 5.6) |
| height | 1.0 ( 0.6) | 0.0 ( 0.0) |
| Nostrils: distance between inner ends | 4.0 ( 2.5) | 3.0 ( 1.6) |
| Labial furrow length from angle of upper jaw | 8.0 ( 5.0) | 11.0 ( 5.8) |
| Gill opening lengths: 1st | 2.0 ( 1.2) | 2.0 ( 1.1) |
| 2nd | 2.0 ( 1.2) | 2.0 ( 1.1) |
| 3rd | 2.0 ( 1.2) | 2.0 ( 1.1) |
| 4th | 1.5 ( 0.9) | 1.5 ( 0.8) |
| 5th | 1.0 ( 0.6) | 1.0 ( 0.5) |
| First dorsal fin: vertical height | 2.5 ( 1.6) | 4.0 ( 2.1) |
| length of base | 2.5 ( 1.6) | 6.0 ( 3.2) |
| Second dorsal fin: vertical height | 3.3 ( 2.0) | 4.0 ( 2.1) |
| length of base | 14.0 ( 8.7) | 7.0 ( 3.7) |
| Caudal fin: upper margin | 25.0 (15.5) | 27.0 (14.3) |
| lower anterior margin | 20.0 (12.4) | 20.0 (10.6) |
| Pectoral fin: outer margin | 16.5 (10.2) | 16.0 ( 8.5) |
| inner margin | 10.5 ( 6.5) | 11.0 ( 5.8) |
| distal margin | 13.0 ( 8.1) | 6.8 ( 3.6) |
| Distance from snout to: 1st dorsal | 84.0 (52.2) | 115.5 (61.1) |
| 2nd dorsal | 108.0 (67.1) | 136.5 (72.2) |
| upper caudal | 137.0 (85.1) | 164.0 (86.8) |
| pectoral | 40.0 (24.8) | 42.0 (22.2) |
| ventrals | 94.0 (58.4) | 122.5 (64.8) |
| Interspace between: 1st and 2nd dorsals | 21.0 (13.0) | 16.7 ( 8.8) |
| 2nd dorsal and caudal | 16.0 ( 9.9) | 20.5 (10.8) |
| Distance from origin to origin of: pectoral and ventrals | 56.5 (35.1) | 84.5 (44.7) |
| ventrals and caudal | 41.0 (25.5) | 41.0 (21.7) |

* Female, 161 millimeters in total length.
† Female, 189 millimeters in total length.

1951, we believe that our specimen is the third example of the genus (monotypic) known from the Pacific and the second example in an American collection.

2. **Isistius brasiliensis** (Quoy and Gaimard)

One male specimen (USNM No. 164173), 165 mm. in total length. Collected March 2,

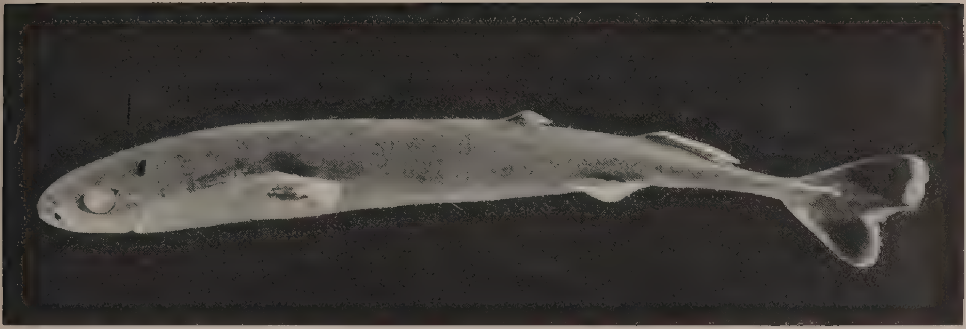

FIG. 1. Lateral view of a 161 millimeter (female) *Euprotomicrus bispinatus* captured at the surface with a dip net at 18°24′N., 175°12′W.

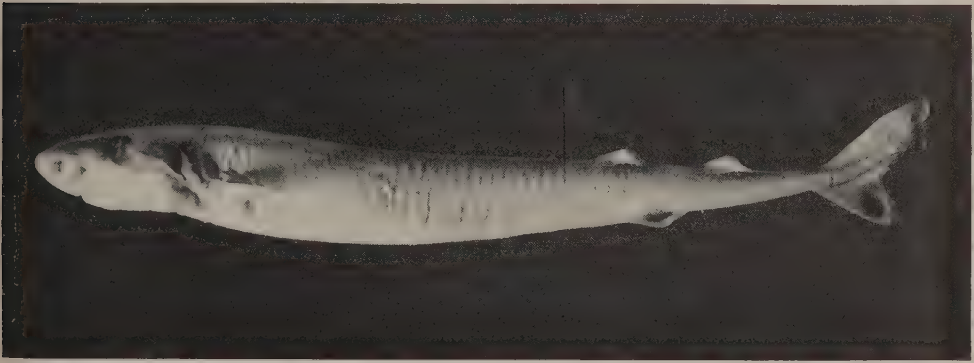

FIG. 2. Lateral view of a 189 millimeter (female) *Isistius brasiliensis* captured by pelagic trawl at 2°09′N., 158° 14′W.

1952, at about 11 P.M. aboard M/V "Hugh M. Smith" at 2°04′S., 168°57′W., in water about 3,000 fathoms deep. Collected by Heeny Yuen employing a 1-meter (mouth diameter) zooplankton net in an oblique haul to 200 meters depth.

A female specimen (USNM No. 164175), 179 mm. in total length. Collected May 23, 1954, at about 8:30 P.M. aboard M/V "John R. Manning" at 4°47′N., 161°04′W., in water about 2,000 fathoms deep. Collected by J. E. King and T. S. Hida in a 6-foot Isaacs-Kidd trawl; depth of haul about 100 meters.

A second female (Fig. 2) (USNM No. 164174), 189 mm. in total length. Collected June 2, 1954, at about 8:30 P.M., aboard M/V "John R. Manning" at 2°09′N., 158°14′W., in water about 1,000 fathoms deep. Collected by J. E. King and T. S. Hida in a 6-foot Isaacs-Kidd trawl with depth of haul about 100 meters. Measurements of this specimen are given in Table 1.

*Description:* No anal fin; 2 dorsal fins, without spines; snout blunt and broadly rounded; 5 gill openings, all anterior to pectorals; body nearly cylindrical; eyes lateral, without nictitating membrane; a large spiracle present; teeth with only 1 cusp and faint indications of serration; teeth of the upper and lower jaw very unlike, the upper are slender and curved outwards while the lower are much larger and erect, with subquadrate bases and triangular sharp-pointed cusp; caudal axis scarcely upturned; subterminal notch in caudal fin slight; mouth transverse, with fleshy lips and a groove extending backwards and outwards from each end; unlike *E. bispinatus*, the first dorsal fin is displaced backward so that the end of its base is over the origin of the pelvic base; dorsal fin bases nearly equal; denticles quadrate with a median pit; first dorsal fin very much nearer pelvics than pectoral base; body dark brown above (in alcohol after fixation in formalin), pale grayish brown below except for a dark collar across the throat; fins with outer edges clear except the upper lobe of the caudal, which is brown to the margin.

This small oceanic shark, although uncommon, is not nearly as rare as *E. bispinatus*. According to Bigelow and Schroeder (1948), *I. brasiliensis* is distributed throughout the tropical and subtropical belts of all three oceans; however, records of its occurrence in the eastern and central Pacific are few. One specimen was taken by the "Albatross" (Garman 1899) at 2°34′N., 92°06′W. F. D. Bennett (1840) captured two specimens, which he classified as *Squalus fulgens*, in a net towed at the surface; one of these, 10 inches in length, he reported as taken at 2°30′S., 163° W., and the other, 18 inches in length, at 55°N., 110°W. Garman (1899) pointed out the obvious error in the latter position and stated that the actual longitude was 140°W. Later G. Bennett (1860) collected a 5.5-inch specimen, which he classified as *Scymmus* sp., at 2°15′S., 163°W. Jordan and Jordan (1922) report the occurrence of this small shark in Hawaiian waters under the name *Apristurus spongiceps* (Gilbert). Fowler and Ball (1925) state that a specimen classified earlier (Fowler 1923) as *Echinorhinus brucus* (Bonnaterre) in Bernice P. Bishop Museum collections from Hawaiian waters is actually *I. brasiliensis*. All the above names are placed in synonymy with *I. brasiliensis* by Bigelow and Schroeder (1948).

The Bennetts' description of the luminiscence of this fish has been quoted often. This characteristic was not observed in our three specimens, possibly because all three were dead when brought on deck. The luminiscence is reported to fade out as the fish dies.

## LOPHOTIDAE

### 3. **Eumecichthys fiski** (Günther) Regan

One specimen (Figs. 3 and 4) (USNM No. 164170), 598 mm. in standard length. Collected June 1, 1954, at about 12:15 P.M. aboard M/V "John R. Manning" at 3°04′N., 159°13′W., in water about 900 fathoms deep. Dip-netted at the surface by Howard Kamauu, fisherman on the "Manning."

*Description:* Body elongate, strongly compressed, nearly of uniform depth throughout, scaleless, snout prolonged as a sword-shaped process bearing near its tip a pronounced plume or crest (Fig. 3); dorsal fin commences at the tip of this process with an extremely long and compressed ray, then continues to the caudal averaging in height about one-fourth the body depth; both jaws are armed with small, hooked teeth; premaxillary nonprotractile; eye large; no ventral fins; anal fin rudimentary, base about 3 mm., height less than 1 mm. (Fig. 4); body silvery (in alcohol after fixation in formalin) with about 30 dark bars or blotches, most prominent dorsally; occipital crest and dorsal and caudal fins were crimson in life but faded completely in formalin.

*Fin ray count:* D. 326; P. 13(R), 12(L); C. 10.

Body measurements (in millimeters) and proportional measurements (bracketed) as percentage of standard length: snout to vent, 579.0 (96.7); vent to base of caudal, 20.5 (3.4); caudal fin length, 25.5 (4.3); head length including snout, 64.0 (10.7); snout length in front of mouth, 30.0 (5.0); snout to origin of pectoral, 64.0 (10.7); greatest body height, 21.3 (3.6); greatest body breadth, 7.0 (1.2); pectoral fin length, 13.0 (2.2); pectoral fin base, 5.0 (0.8); dorsal fin height at mid-point of body, 6.0 (1.0); dorsal fin

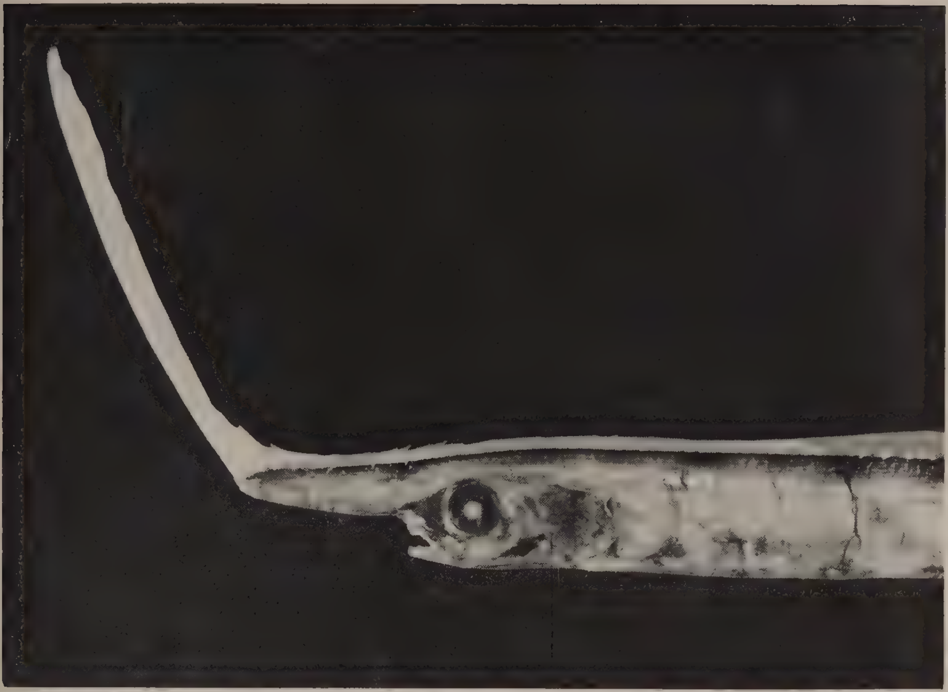

FIG. 3. Head portion of a 625 millimeter specimen of *Eumecichthys fiski* captured at the surface in a dip net at 3°04′N., 159°13′W.

height at origin of pectoral, 4.0 (0.7); dorsal fin height above vent, 5.0 (0.8); crest height, 83.0 (13.9); eye diameter, 8.0 (1.3).

Details of the capture of this rare fish are perhaps of interest. About midday shortly after starting to haul longline, we ( J. E. King and T. S. Hida) noticed a thin "shadow" near the surface just to the rear of the point where the line was emerging from the water. When we finally decided that this was not just the wake or the shadow of the line but an unusual object in the water, a dip net was hastily obtained and this very rare fish was scooped from the water. After it had lain on a canvas hatch cover for a few minutes, we noticed that a black fluid had been discharged from the vent. Kershaw (1909) and Griffin (1934) have reported that lophotid fishes may emit an inky fluid from the vent when captured.

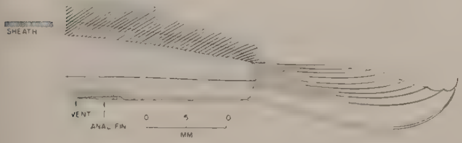

FIG. 4. Tail portion of *Eumecichthys fiski*, same specimen as in Figure 3, with the caudal fin expanded to show the detailed structure of the fin.

*Eumecichthys fiski* was described by Günther (1890) as *Lophotes fiski* from a 50-inch specimen that washed up on the shore of Kalk Bay (Atlantic Ocean), South Africa. In this fish the caudal region was mutilated so that Günther did not know the nature of the caudal fin nor whether or not an anal fin was normally present. Apparently no additional examples had appeared in South Africa by the time of the recent revision of "The Sea Fishes of South Africa" by Smith (1953). From our examination of available literature, we conclude that all subsequent collections of *E. fiski*, except for our specimen from the central Pacific, have been from Japanese waters. Kamohara (1949) states that "In Japan a specimen 88.1 cm. in length was collected off Kōchi in 1939 by a worker from the Kōchi Prefectural Fisheries Experiment Station and was reported by Dr. Kiyomatsu Matsubara. This was the first record from Japan. Thereafter it transpired that several specimens had been captured in 1937 off Hagi in Yamaguchi Prefecture, and that it had also been collected earlier in Wakayama Prefecture." [Translated from the Japanese by W. G. Van Campen.] There is no reference to this species in *The Fishes of Oceania* (Fowler, 1928) and its supplements, or in *The Fishes of the Indo-Australian Archipelago*, Vol. V (Weber and De Beaufort, 1929), which includes the Allotriognathi.

## TRACHYPTERIDAE

### 4. ? **Trachypterus iris** (Walbaum)

Two specimens (USNM No. 164172), both juveniles; one 84 mm. in standard length (caudal fin broken), the other 109 mm. in standard length (146 mm. total length with caudal depressed to the horizontal). Collected May 4, 1953, at about 8 P.M. aboard M/V "John R. Manning" at 8°32′N., 150°12′W., in water about 2,700 fathoms deep. Collected by W. F. Royce and W. M. Matsumoto in a 10-foot Isaacs-Kidd trawl; depth of haul about 200 meters.

*Description:* Body elongate, strongly compressed, scaleless; dorsal fin extends length of body; caudal fin divided, the larger portion pointing upwards; rays of dorsal bear numerous spinules; lateral line spinous; vent located on mid-ventral line in contrast to the following species; anterior profile steep, almost vertical; mouth protractile; body pale colorless (in alcohol after fixation in formalin).

*Fin ray count:* D. 160 ± 2; P. (broken); V. 5; C. 7 (upper), 5 (lower).

Body measurements (in millimeters) and proportional measurements (bracketed) as percentage of standard length (109 mm.):. snout to vent, 70.0 (64.2); vent to base of caudal, 42.0 (38.5); caudal fin length, 34.0 (31.2); greatest body height, 18.0 (16.5); greatest body breadth, 6.0 (5.5).

### 5. ? **Trachypterus woodi** Smith

One specimen (Fig. 5) (USNM No. 164 171), a juvenile, 113 mm. standard length (125 mm. in total length with caudal depressed to the horizontal). Collected May 25, 1954, at about 8:30 P.M. aboard M/V "John R. Manning" at 4°41′N., 159°53′W., in water about 2,000 fathoms deep. Collected by J. E. King and T. S. Hida in a 6-foot Isaacs-Kidd trawl; depth of haul about 100 meters.

*Description:* Body strongly compressed; dorsal fin extends length of body; rays of dorsal with numerous spinules, each ray with a pair of larger spines near its base; rays at anterior end of dorsal show evidence of having been prolonged but are now broken; anterior profile steep but less than vertical; body covered with small, smooth tubercles, particularly prominent in the ventral region; lateral line spinous; no anal fin; caudal fin turned upwards, with no downward projecting portion as in previous specimen; vent located on left side of body about 2 mm. above mid-ventral line (the asymmetrical location of vent in *T. misakiensis* was pointed out by Herre and Herald in 1950); body silvery (in alcohol after fixation in formalin) with scattered dark blotches below dorsal and perhaps seven small blotches extending posteriorly in a line beginning at the upper margin of the eye; fins pink in life, clear in alcohol.

*Fin ray count:* D. 144, P. 12, V. 9, C. 8.

Body measurements (in millimeters) and proportional measurements (bracketed) as percentage of standard length: snout to vent, 78.5 (69.5); vent to base of caudal, 34.0 (30.0); caudal length, 12.5 (11.0); head length, 22.0 (19.4); eye diameter, 7.3 (6.4); snout to origin pectorals, 19.0 (16.7); snout to origin ven-

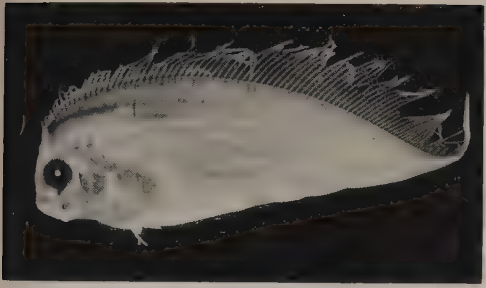

FIG. 5. Lateral view of ?*Trachypterus woodi* Smith captured by pelagic trawl at 4°41′N., 159°53′W.

trals, 23.0 (20.3); greatest body height, 38.0 (33.5); greatest body breadth, 8.0 (7.0); greatest height of dorsal fin, 18.0 (15.9).

Most of the trachypterids examined by ichthyologists have been washed up on shore and usually were in poor condition; our specimens are among the few to be collected in nets, and one of the three, at least, is in excellent condition. These fishes are noted for their change in body proportions during development after the postlarval period (Hubbs, 1926). The juveniles are particularly difficult to identify. On the basis of available literature we have decided that two of our specimens are most likely *T. iris* (Walbaum), which is principally a Mediterranean species, whereas the third resembles *T. woodi* Smith. Although the identification is not certain due to limited library facilities and lack of sufficient reference material, our description, measurements, and the accompanying figure may provide some worthwhile information to those who are interested in the life history of these unusual fishes.

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# BODY-SCALE REGRESSIONS IN JUVENILE CUTTHROAT FROM YELLOWSTONE LAKE

**Martin Laakso**
**U. S. Fish and Wildlife Service, Logan, Utah**

FISHERY biologists have used various methods to distinguish discrete fish populations. Lea (1929) suggested that growth characters might be used as a basis for identifying races. He and his associates found that scales of the Norwegian herring, *Clupea harengus* Linneaus, exhibit differences in growth types associated with place of origin and stock to which an individual or a population belongs. Ford (1933) used regression equations to express differences in growth of herrings from different areas. He found that slope values based on calculated lengths in successive years increased from south to north. Felin (1951) used Walford's (1946) method of growth curve transformation in studying the effect of temperature differential on growth of *Platypoecilus maculatus* Gunther. She found that the growth rate was not affected by temperature differences and apparently served as a useful physiological character descriptive of a population of fishes. Cope (1956) analyzed evidence that indicated distinct races exist in the Yellowstone cutthroat trout, *Salmo clarki lewisi* (Girard), population. Since it appeared that additional evidence could be obtained from available collections of juvenile trout scales collected at several localities, a comparative study of body-scale relations was undertaken.

The material used in the present study consisted of scale mounts prepared at the U. S. Fishery Station at Yellowstone Park by Fish and Wildlife Service personnel. The collections were made by electro-shocking, seining, poisoning, and dip-netting immature cutthroat trout from tributary streams and the shores of Yellowstone Lake in 1953.

Scales from 196 fingerlings were examined and measured with the aid of a micro-projector under a magnification of 110. The fingerlings in all of the samples were less than 70 millimeters long and therefore were largely age-group 1 except for the fall-caught sample which was assumed to be group 0 (table 1).

Slides were prepared by scraping scales from the area between the lateral line and the dorsal fin and mounting them in glycerine-gelatin medium. It was found that scales displayed considerable size variation on each mount. Owing to the difficulty of deter-

Table 1. *Average scale diameters of immature cutthroat trout from various localities on Yellowstone Lake*

| Locality | Date | Midpoints of length groups | | | | | | | | | Total number |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 44 | 47 | 50 | 53 | 56 | 59 | 62 | 65 | 68 | |
| Arnica Creek | V- 5-53 | 9.0(2)* | 13.5(2) | 14.5(2) | 14.8(8) | 16.5(4) | 19.0(4) | 19.6(5) | 21.0(4) | 22.7(3) | 36 |
| Fishing Bridge | V-11-53 | 10.7(10) | 13.4(12) | 15.0(8) | 18.9(14) | 20.0(1) | 24.3(4) | | | | 49 |
| Hatchery Creek | V-11-53 | 10.0(1) | 13.1(6) | 16.4(5) | 16.3(3) | 18.2(5) | 18.3(4) | 19.8(5) | 23.0(2) | 22.7(3) | 33 |
| Bridge Bay | V-17-53 | | | 15.0(2) | 16.3(4) | 20.3(4) | 19.3(3) | 21.7(7) | 23.0(3) | 23.6(5) | 28 |
| Fishing Bridge | X-23-53 | 12.0(2) | 13.9(10) | 18.1(14) | 19.5(10) | 22.0(6) | 23.3(8) | | | | 50 |
| Mean scale diameters | | 10.4 | 13.5 | 15.8 | 17.2 | 19.4 | 20.8 | 20.4 | 22.3 | 23.0 | 196 |

*Numerals in parentheses indicate numbers of specimens

Table 2. *Calculated 't' values obtained by comparing body-scale regression coefficients for five groups of data*

| | | Fishing Bridge V-11-53 | Arnica Creek V-5-53 | Hatchery Creek V-11-53 | Bridge Bay V-17-53 |
|---|---|---|---|---|---|
| Fishing Bridge | X-23-53 | 0.40 | 2.52** | 1.88* | 2.67** |
| Fishing Bridge | V-11-53 | | 3.75*** | 2.37*** | 4.68*** |
| Arnica Creek | V- 5-53 | | | 0.12 | 0.21 |
| Hatchery Creek | V-11-53 | | | | 0.01 |

* Significant at 10 percent level
** Significant at 5 percent level
*** Significant at 1 percent level

mining their mean size accurately, the data represent measurements of the largest symmetrical scale on each slide. The scale image diameter measured on the longest axis was recorded to the nearest tenth inch for each fingerling in the various collections. Data for each locality were grouped into 3 millimeter total length intervals. The weighted mean scale size in each class was plotted against the class midpoint representing average body length. Regression lines were then fitted for each group of points by the least squares method. Significance of differences between regressions was tested by calculating 't' values for each pair of regression coefficients (Simpson and Roe, 1939).

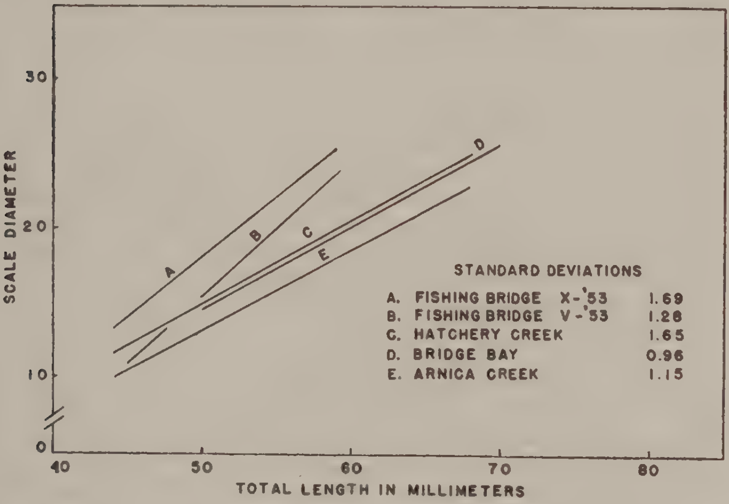

Fig. 1. *Regressions of scale diameter on total length for immature cutthroat trout from four localities*

When mean scale diameter was plotted against total length in the various collections, a nearly linear distribution of points resulted in each case. On the basis of the apparent straight line trends, the limited range of fish sizes involved, and the high correlation coefficients of the regressions (range: 0.90-0.98), linear equations were calculated for each group of data. The fitted lines (figure 1) divide into two statistically homogeneous groups. Intergroup comparisons exhibited differences significant at the 5 and 1 percent level, except in the comparison between Fishing Bridge on October 23, 1953, and the Hatchery Creek sample. This was significant at the 10 percent level (table 2).

In order to measure the variability of the scale diameter about the line of best fit, the standard deviation of the regression (standard error of estimate) was calculated for each line. The low values (figure 1) indicate that the average scale lengths deviated little about the lines of central tendency. The upper two regression lines describe the data for samples taken near Fishing Bridge. These data represent successive year-classes of fingerlings captured close to the outlet of Yellowstone Lake. The remaining three collections captured in Arnica Creek, Bridge Bay, and Hatchery Creek, respectively, were taken in small tributaries or along the adjacent lakeshore. The greater slope values for the fish from Fishing Bridge (0.73-0.91) compared to values for specimens from the small tributaries (0.49-0.52) suggest that racial differences distinguish the two groups of fingerlings.

For some time, biologists have recognized color differences between fish caught in Yellowstone River and those taken in the lake. Cope showed more conclusively by means of data on average total lengths, egg numbers and sizes, ovary characters, and spawning periods that several independent races comprise the Yellowstone population. Homing evidence by Cope and also by Ball (1955) shows that cutthroat return to spawn in their parent stream; therefore, the trout associated with each stream constitute a discrete reproductive unit.

While genetic factors may account for the discrepancies in scale growth, the possible influence of ecological factors should also be considered in this connection. Physical, chemical, and biotic factors differentiate the lake-type environment at the river outlet from conditions in the small tributaries. While Arnica Creek, Bridge Bay Creek, and Hatchery Creek differ from each other in mean temperatures, volumes, gradient, and good production it is undoubtedly true that growth of resident fish in any one of them would be appreciably slower than in the lake. Evidence based on calculated growth rates of Arnica Creek residents and fish from the lake supports this view. The discrepancy in body-scale regressions therefore could possibly be attributed to ecological factors. However, Felin's work on growth in the presence of temperature differences suggests that such is not the case. Selectivity in sampling may also account for the difference between the "river" and "stream" body-scale ratios. The question arises whether the samples taken in the small tributaries in May have growth characteristics similar to those that move down to the lake during the previous late summer and fall. The fingerlings collected in the spring might perhaps differ from the total population mean.

It is expected that future improved collecting techniques will yield more reliable samples to permit a better understanding of the factors affecting relative growth during early periods of life.

## Summary

The body-scale regressions of juvenile cutthroat trout from different areas in Yellowstone Lake showed significant differences in slope. Curves plotted for fingerlings captured near the outlet of Yellowstone Lake exhibited steeper slopes, showing that scale growth was relatively faster than in fish collected along the lakeshore and in two small tributaries.

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# AGE DETERMINATION IN YELLOWSTONE CUTTHROAT TROUT BY THE SCALE METHOD

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ge determination of various subspecies cutthroat trout has posed a problem for ery workers in the West for many years. cognition of annuli has been somewhat re difficult than in trouts forming larger nbers of circuli, and obvious discrepancies e appeared when workers attempted to te numbers of annuli to actual ages. is difficulty has resulted in a general dency on the part of biologists to avoid ding cutthroat trout scales. No other thod of aging this species has been ex- red extensively.

iologists of the U. S. Fish and Wildlife vice faced the problem of aging Yellow- ne cutthroat trout (*Salmo clarki lewisi*) en they began investigations on that cies in Yellowstone Lake in 1950. The llowstone studies have been concerned h determining safe fishing pressure for llowstone Lake and the Yellowstone er. The investigation has had several ets, and progress has been reported oore, Cope, and Beckwith, 1952; Cope, 3). The scale-collecting program pro- ded according to schedule from year to r, but scale reading lagged because of iculties in assigning ages to the different .

he present paper explains the methods eloped for age determination in cut- oat trout from Yellowstone Lake and necting waters. The biology of the fish its first year has proven to be variable its effect on the scale, and the principal blem in age determination lies in the in- pretation placed on the structures in the ter of the scale.

Lake Yellowstone, at an elevation of 7,731 feet, has an area of 137 square miles, and an irregular shoreline 100 miles long. It extends 20 miles in a north-south direction and 14 miles from east to west (Fig. 1).

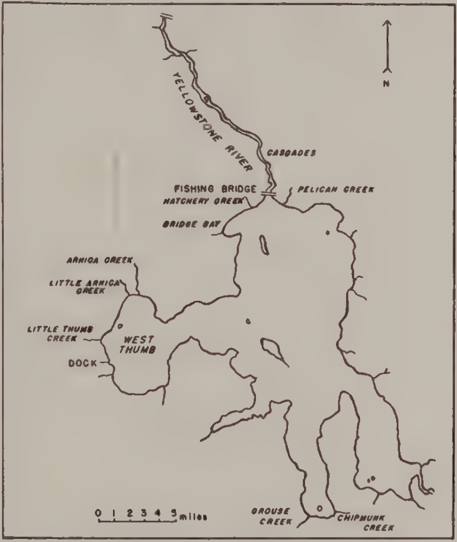

FIG. 1. Yellowstone Lake and lower Yellowstone River, showing streams from which cutthroat trout have been collected.

The Absaroka Range drains into the lake on the east, and the Upper Yellowstone and the Thorofare Creek drainages empty into the south end of the lake.

Ice covers the lake about six months of the year. Mean monthly air temperatures range from 10.7°F. in January to 59.3°F. in July. The mean annual temperature recorded at Lake Ranger Station is 32.5°F. Precipitation is a little heavier during the first six months than during the latter half of the year. Annual precipitation averages 20.16 inches.

Yellowstone Lake cutthroat trout have a

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prolonged spawning period which begins in May and lasts until late July. Fry begin to emerge from the gravel late in June, and the hatch continues until early September. Since the growth rate declines to negligible values in October, (Brown and Bailey, 1952) late fry do not reach the size at which scale formation is initiated. Those reaching a length of 41 to 44 millimeters usually have distinguishable scale platelets. Scales appear first along the lateral line and develop progressively dorsad and ventrad as determined for this species by Brown and Bailey (*op. cit.*) and for other species by various authors. Since the center of most rapid growth shifts caudad during an early stage of development, the peduncle is fully scaled earlier than the medial parts of the body. In order to make age determination as accurate as possible for fish whose early growth is so variable, it is necessary to devise a method for identifying scales which did not form an annulus after the first season of growth.

Definite criteria have been established for identification of "normal" as contrasted with "retarded" scales. Normal scales are defined as those with growth checks at the end of the first growing season, and retarded scales are those that fail to form a check at that time.

A limited amount of work has been done on the biology of the Yellowstone cutthroat trout at Yellowstone in other places. Robertson (1947) investigated the cutthroat trout population of two high-mountain lakes in the Wind River Range, Wyoming. Fleener (1951) studied the life history of cutthroat trout in Logan River, Utah, where they occurred mostly in Age-groups I and II in the headwaters. Brown and Bailey (*op. cit.*) determined the time and pattern of scale formation for a sample of 200 immature cutthroat trout in a tributary of Yellowstone Lake in 1949. Irving (1953) explored the ecology of the species in Henrys Lake, Idaho, a few miles west of Yellowstone Park. Two studies on the Lahontan cutthroat trout, *Salmo clarki henshawi* have been conducted in California, where Smith (1941) investigated natural spawning success at Convict Creek, and Calhoun (1944) reported on spawning and reproduction at Upper Blue Lake. Willoughby (1951) analyzed the age and growth of cutthroat trout obtained from the 1949 spawning runs at Pelican and Chipmunk Creeks, tributaries to Yellowstone Lake.

## COLLECTION, PREPARATION, AND EXAMINATION OF SCALES

Scales for study were obtained from trout taken in the fishery, from spawners entering tributary traps, and from immature fish captured in streams and along the lakeshore. Sampling of the fishery was begun with the opening of the fishing season on May 30, from 1950–1952. In 1953, the opening date was postponed until June 15 thereby delaying the collection of data.

The operation of traps in several streams during early May made it possible to intercept early spawners. These early collections, principally from Pelican, Arnica, Clear, Grouse, and Chipmunk Creeks (Fig. 1) are especially valuable for studies on time of resumption of growth. Scales were collected routinely from these streams, as well as from Cub Creek, during several entire seasons. The data from Arnica Creek were extensive for both upstream- and downstream-migrating adults. A program of fin clipping and scale collecting from lakeward-migrating immature fish in Arnica Creek produced valuable information pertaining to early life history. This marking program also provided adult fish of known age from the returns of marked fingerlings to their parent streams. Scales from 56 of these known-age fish were used for reference in age determinations. Only spawners were taken in the stream traps. Non-spawners and sexually immature fish were not available until later in the year, since they come principally from anglers' creels.

Other cutthroat trout for scale samples came from a Lake Erie type trap-net fished in Yellowstone Lake just southeast of the Yellowstone River outlet. Trap-net collections included smaller, younger fish than the spawning runs. Several hundred upstream migrants were tagged annually at the Yellowstone River Cascades (5 miles below the lake outlet), dip nets being used to capture the fish as they congregated in pools. Recoveries of marked fish from the extensive tagging program on the lake have yielded information on the biology of the cutthroat trout.

Scale collections from the lake and river fisheries were made periodically throughout the season. In 1953, immature trout were captured from the lakeshore, river, and tributary streams for information on scale development in their first and second years of life. Some immature fish were taken October 23, 1953, for data on the late-season development of these fish.

Scales from adult fish were taken from the area between the dorsal fin and the lateral line. Scales from immature trout were obtained both from the area between the dorsal fin and the lateral line and also from the caudal peduncle for the purpose of measuring the differences in scale development in the two areas. After cleaning, four typical scales were mounted on glass slides in glycerin-gelatin or gum arabic medium.

A Bausch and Lomb Euscope was used for viewing scales. The apparatus consisted of a 60-power compound microscope equipped with a prism above the ocular. A light-tight box 12 inches long, fitted with a 6-inch screen was mounted above the microscope. The proximal end of the box was fashioned to fit the face and was equipped with a rectangular lens for magnifying the scale image. The equipment provided good definition without discomfort to the reader even with continuous use. All scales were read twice; the more questionable ones were discarded. Aging was verified by periodic checking by another scale reader. Photographs of scales were made with a microprojector.

## NORMAL AND RETARDED ANNULUS FORMATION

The streams entering Yellowstone Lake vary in quantity and quality of spawning gravel, in gradient, in water volume, in fluctuations of flows, in temperature, in abundance of food organisms, and in chemical characteristics. Temperatures and chemical characteristics appear to exert the greatest influence on growth and scale development. In some areas geysers and hotsprings empty their hot, chemically-charged effluents into streams. For example, fish are prevented from entering sections of Pelican and Clear Creeks in the vicinity of geyser basins through which they flow. It is presumed that failure of immature fish to migrate at normal times retards growth and scale development. These unfavorable stream conditions are exaggerated during periods of high isolation and reduced precipitation.

The survival of a particular spawning, during and after the period of incubation, is conditioned by the stream and climatic conditions. The variability in size of fingerlings in the fall of any brood year reflects the extent to which environmental conditions were favorable during the summer. For example, if fish with retarded scales constitute an abnormally large percentage of the year class, it is presumably the result of greater survival of the progeny of late spawning. Observations made during 1954 stream surveys indicated that, in September, young of the year were of greater average size in Pelican than in Cub Creek. This difference can be attributed to the later spawning run and lower stream temperature in Cub Creek.

Brown and Bailey (1952) divided first-year fish into the following groups on the basis of scale development:

1. Fish completely scaled before the first winter and having the first year mark on their scales.
2. Fish partially scaled before the first winter, having some scales with, and some scales without, the first year mark.
3. Fish which have no scales before the first winter, and hence form the first annulus only after the second growing season.

As used in the present paper, partially scaled fish were defined as those exhibiting three or less circuli at the end of the first growing season. Fish in this group appeared to form annuli only after the second growing season. The 1953 collections of scales from immature fish were examined with a view toward determining the frequencies of occurrence of the developmental groups listed above. The results of the examination of 272 fish assumed to belong to Age-group I are shown in Table 1. The group classed as completely or almost completely scaled by the end of the first growing season were identified by the presence of four to six circuli. This criterion was established by repeated observations on immature and adult trout, which indicated that almost without exception there was a minimum of four circuli within the first recognized annulus. The partially scaled group was most plentiful in the 1953 example (35.8 per cent of the scales examined), the unscaled group had the second greatest occurrence (32.7 per cent), and the fully scaled group averaged 31.5 per cent of the sample (Table 1). The collections may not have been representa-

TABLE 1.—SCALE DEVELOPMENT AND LENGTHS OF AGE GROUP I FISH COLLECTED IN 1953

| | | Unscaled | | | | Partially Scaled (1–3 Circuli) | | | Fully Scaled (4–6 Circuli) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Locality | Date of Capture | Number | Percentage | Mean length* | Range in length | Number | Percentage | Mean length | Number | Percentage | Mean length |
| Arnica Creek.... | May 5, 1953 | 5 | 23.8 | 43.0 | 40–46 | 9 | 42.9 | 50.5 | 7 | 33.3 | 51.3 |
| Little Arnica Creek......... | May 5, 1953 | 13 | 76.5 | 37.5 | 31–46 | 3 | 17.7 | 51.3 | 1 | 5.9 | 60.0 |
| Below Fishing Bridge........ | May 11, 1953 | 4 | 6.8 | 41.5 | 40–43 | 30 | 50.9 | 47.5 | 25 | 42.3 | 54.7 |
| Hatchery Creek . | May 11, 1953 | 4 | 30.8 | 40.8 | 36–44 | 7 | 53.9 | 45.9 | 2 | 15.3 | 48.0 |
| Little Thumb Creek......... | May 13, 1953 | 40 | 62.5 | 41.6 | 35–48 | 19 | 29.7 | 51.8 | 5 | 7.8 | 56.2 |
| Bridge Bay...... | May 17, 1953 | 1 | 6.3 | 44.0 | ..... | 2 | 12.5 | 50.5 | 13 | 81.2 | 57.7 |
| Fishing Bridge Dock......... | Oct. 23, 1953 | 18 | 22.0 | 39.9 | 35–44 | 35 | 42.7 | 48.0 | 29 | 35.3 | 55.9 |
| MEAN VALUES | | | 32.7 | 41.2 | | | 35.8 | 49.4 | | 31.5 | 54.8 |

* Average total length in millimeters.

tive of the entire stock since only seven localities were sampled.

Most of the fingerlings were taken in May, but a sample of 82 was obtained on October 23. This fall collection was judged to be composed entirely of young of the year on the basis of size distribution and scale appearance. Squamation was evident on 78 per cent of the collection which ranged from 35 to 65 millimeters in total length. The appearance of their scales was characterized by extreme transparency and by the uniform lack of contrast exhibited by circuli. Further calcification presumably occurs during the winter, since the scales of Group I fish of the same average size taken in the previous May, were more refractive. The number of circuli varied from 0 to 6, the smallest fish with discernible dorsal scales being 41 millimeters in length. Four out of ten specimens in the 41 to 43 millimeter range yielded scales. Most of the fingerlings measuring 45 to 50 millimeters had 1 to 3 equally spaced circuli. Larger ones generally had scales with 4 to 6 circuli of which the marginal ones were crowded together in the anterior field to form the year mark. Initial circuli enclosed areas up to 0.5 inch in longest diameter irrespective of scale size (magnification 110×).

The scales of 59 fingerlings taken in an adjacent area May 11, 1953, were found to be comparable in size and numbers of circuli to the October collection. In both of the groups there were from 0 to 6 circuli. The size range of the fish in the May collection from Fishing Bridge was 40 to 65 millimeters—very similar to the size distribution of the fall sample except for the presence of a greater number of unscaled fingerlings in the latter group. Scale development appeared to be comparable for equal-sized fish in both collections. The scales of the specimens were measured and circuli enumerated. The data were combined and mean scale length was plotted against total length by 20 millimeter intervals in order to show the body-scale relationship of fish at the end of the first growing season (Fig. 2). Since the evidence indicated that

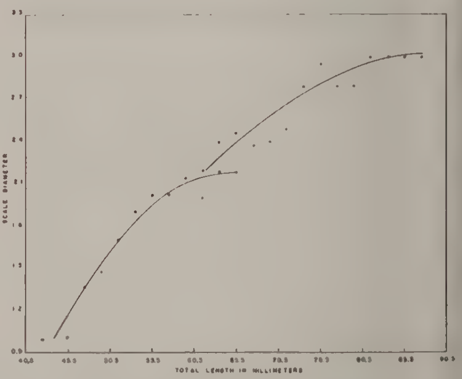

FIG. 2. Body-scale relationship of immature Yellowstone cutthroat trout. Date for lower curve obtained from 119 trout presumed to be Group O and Group I. Upper curve based on data from 32 fingerlings presumed to be Group II.

the Fishing Bridge area samples were homogeneous as to age composition, the body-scale relation was based on these fish only, in order to avoid the necessity of selecting young of the year on arbitrarily established bases.

The diameter of the scale along the long axis was measured and the average length of the three largest scales was recorded to the nearest tenth of an inch under a magnification of 110×. A second-degree curve was fitted to the data by Fisher's summation method of fitting orthogonal polynomials (Goulden, 1952) by means of the equation: Scale length $= 1.75 + .05734\xi_1 - .0038145\xi_2$.

The data indicate that the body-scale ratio was nearly linear in the range between 47 and 55 millimeters. Earlier scale growth was slow, and a progressive lag in scale growth is evident in fish above 55 millimeters in length. The fitted curve corresponds generally to curves for adult populations. The second curve plotted in Figure 2 permits a comparison of the Age-groups 0 and I with fish considered to be Group II. The data for the upper curve represents measurements on 32 fingerlings which were believed to belong to the 1951 year class on the basis of more numerous circuli (8 or more, a few had 7 circuli). The fish measured from 62 to 92 millimeters in total length. Initial circuli on about 10 per cent of the sample had a characteristically "open" appearance measuring 0.6 to 0.7 inch in diameter on the long axis. As previously indicated, none of the Group 0 and I fingerlings from the Fishing Bridge vicinity had central areas exceeding 0.5 inch in diameter. It is postulated that scales with the enlarged central area occur on fish which failed to form scales (in the area of skin sampled) until its second year. Annuli of varying distinctness occurred on the scales of 20 per cent of the specimens. The Age-group II fish were seined from the Bridge Bay area where conditions are presumed to be comparable to the environment near Fishing Bridge. The collection is presumed to represent fish of smaller than average size belonging to the 1951 year class. Group II specimens from Arnica and Hatchery Creeks were excluded, since scales from fish in these collections were typically "stream type" with relatively small size and with crowded circuli. The data for body-scale lengths of Group II fingerlings was fitted with a curve by Fisher's summation method (Goulden, *op. cit.*) having the following equation: Scale length $= 2.69 + 0.3301\xi_1 - 0.0592\xi_2$.

The points about the upper curve in Figure 2, representing mean scale length for each interval of total length, display considerable scatter attributable to any of three causes. The possible heterogeneity of the population sampled, possible lack of randomness due to small sample size, and the necessity of using the average of the largest, instead of key scales, may have contributed to the dispersion.

In general, it appears that the relationship of scale and body length tends to be linear in the median range and progresses toward an asymptote near the upper size limit in both age groups. While there is a region of overlap in the body as well as the scale length in the two groups of fish on which the curves are based, the difference in slopes suggests that they probably are different age groups. Measurements and circulus counts on normal and retarded scales of adult fish were also compared (Table 3). Significant differences were found to exist between the two types of scales.

As a result of the prolonged spawning season, fry were in various stages of scale formation at the end of the growing season. Brown and Bailey (1952) found that fingerlings 54 to 61 millimeters long had formed less than half their scale rows above the lateral line during the first summer. The median length of the fingerlings collected in 1953 was about 50 millimeters. These facts suggest that in their first summer a large percentage of all fingerlings can be expected to lay down none or only part of their ultimate scale rows in the area normally sampled for scales. A study of the 1953 fingerlings showed that although some degree of squamation occurred in two-thirds of the fish examined, first-year annuli occurred on only 31.5 per cent of the population (Table 1), since late-hatched fry which form rudimentary scales nevertheless fail to establish a year mark until after the second summer.

The annulus, as it appears in cutthroat trout scales, conforms in a general way to the usually accepted definition. However, the small scale size and the presence of small numbers of circuli in the average year's scale growth, complicates the aging technique. Van Oosten (1929) described the annulus of the lake herring as a band of faint, much broken, anastomosed, and more closely approximated circuli formed during a period of retarded growth. The year-mark on the scale of the cutthroat trout is scarcely a band, or at best is a very narrow one since it usually involves only 2 or 3 circuli. Growth cessation appears to be abrupt, and the broken, anastomosed circuli constituting the year mark occur in the anterior and lateral fields. Occasionally a single faint and irregular circulus indicates the occurrence of the annual growth check. Age group I fish having only 2 or 3 circuli in May, usually have circuli that are equally spaced and of uniform appearance. Although these first-year circuli often appear to be capable of differentiation from later ones on the basis of optical differences, circulus counts seem to be the more reliable single basis for the recognition of retarded annulus formation.

Occasionally, a false annulus is recognizable by the incompleteness of the growth check in the lateral fields and by its occurrence in the area representing summer growth. Since Yellowstone Lake temperatures probably never reach unfavorably high levels, the false checks are probably effects of late spawning, disease, or injury. Temperatures in some streams may reach unfavorable levels for short periods, as at Arnica Creek where afternoon readings were above 70°F. during the latter portion of the 1953 spawning run. This condition may produce false checks on scales. A few fish taken and returned to the water by anglers may suffer injury sufficient to produce a growth check. Irving (1953) reported well-defined false checks on cutthroat trout in Henrys Lake where high temperatures and reduced oxygen tensions coincided with a cessation of feeding during the late summer.

Investigation of the time of annulus formation in the fall is made difficult by waning fishing effort and success. In 1953, the season's last adequate sample at West Thumb was collected on August 25, at which time scales showed no evidence of slowed growth.

### TIME OF ANNULUS FORMATION

Annuli were assumed to have been completed when a continuous new circulus appeared outside the seasonal growth check. Adult fish taken in traps and in the early fishery were examined to determine the presence or absence of current scale growth (Table 2).

Approximately one-half of the fish taken during the first 3 days of the run in Arnica Creek, where the first 1953 migrants entered the trap on May 5, had newly formed circuli on the scale margin, and were therefore considered to have recently formed an annulus.

To determine whether time of resumption of scale growth was conditioned by calendar age, the fish were divided into size groups. Of all specimens examined, annulus formation had occurred in 87 per cent of those up to 13 inches inclusive compared to 50 per

TABLE 2.—PERCENTAGE OF ANNULUS FORMATION, BY AGE-GROUPS, IN YELLOWSTONE LAKE

| Locality | Date | Number of fish sampled | Percentage of scales with annuli | | |
|---|---|---|---|---|---|
| | | | Age groups | | |
| | | | III & IV | V | VI & above |
| Arnica Creek Trap......... | May 5–14, 1953 | 55 | 81 (26)[1] | 41 (17) | 25 (12) |
| “ “ “ ......... | May 18–20, 1953 | 29 | 93 (15) | 71 (7) | 43 (7) |
| “ “ “ ......... | June 1, 1952 | 34 | 85 (20) | 41 (11) | 100 (3) |
| “ “ “ ......... | June 10–13, 1953 | 49 | 100 (7) | 85 (27) | 53 (15) |
| “ “ “ ......... | June 16, 1952 | 16 | 100 (10) | 75 (4) | 100 (2) |
| Pelican Creek Trap......... | May 20, 1952 | 47 | 85 (13) | 12 (25) | 0 (9) |
| “ “ “ ......... | June 10–12, 1953 | 31 | 100 (6) | 100 (10) | 60 (15) |
| Fishing Bridge Dock........ | June 6–9, 1950 | 62 | 80 (20) | 32 (28) | 14 (14) |
| “ “ “ ........ | June 16, 1951 | 23 | 67 (3) | 50 (10) | 30 (10) |
| “ “ “ ........ | June 16, 1953 | 22 | 100 (9) | 22 (9) | 0 (4) |
| West Thumb Dock......... | June 10, 1952 | 23 | 83 (19) | 0 (4) | .... |
| “ “ “ ......... | June 16, 1952 | 17 | 100 (16) | 100 (1) | .... |
| “ “ “ ......... | June 16, 1953 | 20 | 100 (17) | 50 (2) | 0 (1) |

[1] Numbers in parentheses represent numbers of fish.

cent in the group larger than 13 inches. When divided into groups above and below 15 inches total length, current scale growth was evident in 72 per cent of the smaller and 28 per cent of the larger specimens. Hence, seasonal growth resumption was delayed in fish of larger size.

The effect of age on annulus formation is shown in Table 2 which reveals that in the June 16 collection of all years, almost every Group III fish had completed annulus formation. A comparison of spawning migrants with specimens from the fishery (Fig. 3) shows higher percentages of annulus formation in the spawners. The graph suggests that early-season spawners resume scale growth in the spring before late or non-spawning trout do. Almost all 3- and 4-year-old fish completed annulus formation by June 15.

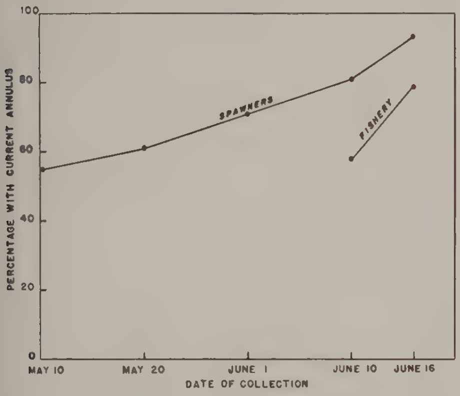

FIG. 3. Percentage of annulus formation in 428 fish of Age-groups III, IV and V in the fishery and spawning runs of 1951–53.

Collections of scales made during the first half of June from 1950 through 1953 were examined for extent of annulus formation by areas. A sample of 160 fish landed at West Thumb and Fishing Bridge docks showed annulus formation in 80 and 50 per cent of the fish, respectively. However, the Fishing Bridge specimens were longer on the average (383 millimeters) than fish of the West Thumb collections (335 millimeters). About 80 per cent of the smaller fish (below 14 inches) in both groups had formed annuli. Brown and Holton (1953) found that annulus formation was completed in young rainbow trout by June 3, and in brook trout by June 25 in Trout Creek, a spring-fed stream located in the Gallatin Valley, Montana.

## CUTTHROAT TROUT SCALES

The difficulties encountered in attempting to read the scales of Yellowstone cutthroat trout led to an evaluation of the unique morphological features of these scales. Special attention was given the structure of the focus, the spacing and numbers of circuli, the appearance of the scale surface, and the structure of the annulus. From this examination were developed standards for determining the age of Yellowstone cutthroat trout.

The part of the scale within the first annulus, and the part outside to a lesser extent, exhibit great variability with respect to several features. The size of the first annulus varies according to the amount of growth in the first year and according to the numbers of circuli within. This point will be discussed later but Yellowstone cutthroat trout scales appear to have from 4 to 16 circuli within the first year mark. The smallest number of circuli within a distinct annulus was four. The spacing of the circuli within the first annulus is remarkably uniform, considering the migratory nature of the fish. Very few scales with a so-called "stream nucleus" are found despite the fact that large numbers of the immature fish overwinter in streams. The examination of more than 1,300 scale samples from the Fishing Bridge and West Thumb areas for narrowly spaced circuli of the familiar "stream nucleus" disclosed only nine examples (0.7 per cent). This type of scale marking was more common in the West Thumb samples, where it occurred in two per cent of the fish examined. The size of the initial circulus is variable from fish to fish, depending upon the time of scale formation. Ordinarily, the central area has a radius very little greater than the distances between circuli in the area representing the first year's growth. Large foci occur in two situations: in the regenerated scale, and where no circuli are formed during the first season. The latter is important for recognition of retarded annulus formation, and will be discussed later. The shape of most foci, with the exception of regenerated ones, is characteristically oval, with smooth, regular contours. Regenerated scales of cutthroat trout have nuclei similar to those of other salmonids, *i.e.*, with irregular shape, large size, and rough outlines on the first circulus. The surface bounded by the first circulus is sometimes seen as a coarse granular area. This condition is usually observed also on fish which fail to form annuli during their first winter.

The number of circuli formed during a year of growth in Yellowstone cutthroat trout scales is not as large as in certain other trout species. A small sample of Age-group II rainbow and cutthroat trout from Koosharem Reservoir, Sevier County, Utah, collected July 26, 1953, were compared as to numbers of circuli formed in the year preceding capture and in the current season. During the summer of 1952, the rainbow and cutthroat trout laid down an average of 19.5 ($\sigma \pm 0.7$) and 9.0 ($\sigma \pm 1.4$) circuli, respectively. By July 26th in 1953 they averaged 12.0 ($\sigma \pm 1.4$) and 6.5 ($\sigma \pm 2.12$) circuli for rainbow and cutthroat trout, respectively. It appeared that in the sample examined, rainbow trout laid down approximately twice as many circuli as cutthroat trout in the same period. Cutthroat trout from other waters have been examined for general comparison with rainbow and brook trout. Scales from small samples of rainbow trout collected from Fish Lake, Utah (elevation 8,600 feet) in 1933, resembled Yellowstone cutthroat trout in that first-year annuli occurred in one-half of the fish examined. (It was assumed that criteria for identifying first-year annuli in cutthroat trout applied to rainbow also.) Brook trout from the same waters showed more uniform first-year growth than did cutthroat and rainbow trout, and averaged 7 and 8 circuli in their first and second years, respectively.

The scales of cutthroat trout collected in 1933 from Scofield and Strawberry Reservoirs in Utah (elevations 7,625 and 5,725 feet, respectively) were examined for differences in scale characteristics. The scale samples from Strawberry Reservoir indicated that annulus formation occurred during the first year in almost every fish. Only about one-fifth of the fish from the higher and more oligotrophic Scofield Reservoir exhibited the first year mark.

In order to obtain a basis for distinguishing the normal and retarded scale types, the scales from Group I and II fingerlings taken in 1950–1953 were studied to determine limits of first- and second-year scale development.

Three scale characters were of value in helping to recognize retarded development:

1. Number of circuli inside the first annulus.
2. Size of the first circulus.
3. Shape and density of the first one or two circuli.

On the basis of information gathered in a study of scales from immature fish, (Table 1, Fig. 2) first annuli preceded by eight or more circuli were designated as second-year marks. Growth checks preceded by six or fewer circuli were considered to indicate termination of first-year growth. Studies by other workers have shown that annual growth increments were smaller at higher elevations. Robertson (1947), working with cutthroat trout of two high-mountain Lakes (10,500 ft.) in Colorado, and Curtis (1935), in a study of golden trout of the Cottonwood Lakes, California (11,000 ft.) both postulated that annuli preceded by five circuli or less were indicative of first-year scale growth. These workers found that scale development which had been delayed until after the first winter was indicated by seven or more circuli within the first growth check.

Scales which had only one or two incomplete circuli at the end of the first growing season were often recognizable in adult trout. Brown and Bailey (1952) pointed out that the first circuli are often incomplete anteriorly. The dark appearance of the first, second, and sometimes the third circulus is characteristic of some cutthroat trout scales and may be the result of growth interruption at one of these stages. Fingerlings which lack expected year marks, but which were presumed to be Age-group II on the basis of size and circulus counts, showed both of these characteristics. Scales from immature specimens collected late in October were examined to determine the extent of current annulus development. Fish mostly less than 50 millimeters in length having scales with not more than three circuli did not exhibit approximation of marginal circuli as evidence of annulus formation. Larger specimens displayed growth retardation at the scale margin as indicated by the crowded condition of the marginal circuli.

Representative scales illustrating types commonly encountered in age analysis were photographed at a magnification of 60× (Figs. 4 to 7). The magnification of figures 8, 9, and 10 was 50×.

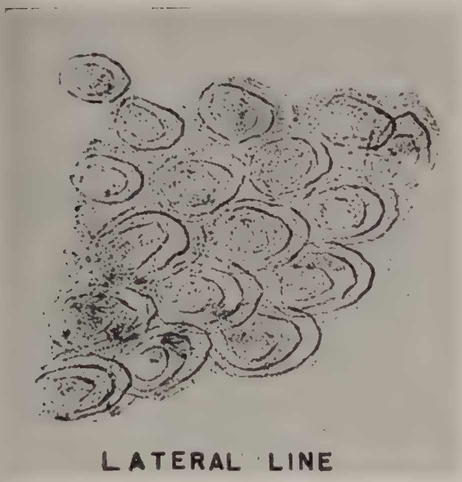

FIG. 4. Group of scales from an Age-group I fingerling showing developmental gradient with circuli increasing from two to four. 50 mm., near Fishing Bridge, May 11, 1953.

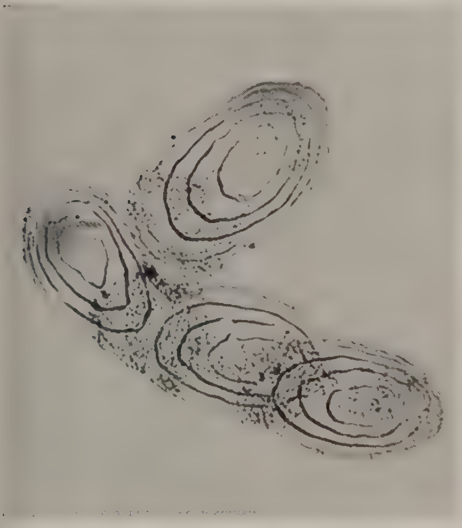

FIG. 5. Beginning of annulus formation in young of the year. 54 mm., at Fishing Bridge Dock, Oct. 23, 1953.

Figures 4 and 5 illustrate moderate scale development in fingerlings less than a year old. In the first photograph, a cluster of scales shows a developmental series with the more advanced scales oriented in the plane of the lateral line. Possible indications of marginal growth retardation by closer spacing of outer ridges occurred only in scales having four circuli. Figure 5 demonstrates slowed fall growth in a more advanced fingerling taken at the end of the first growing season. The scales in Figure 6 were obtained from fingerlings judged to be in their second season on the basis of length and scale characteristics. A poorly defined first-year mark occurs on one scale but the six others generally conform to the retarded type. The larger number of circuli in the scale having an annulus indicates that it was formed earlier, probably during the first summer, while the larger foci and the absence of checks on the others suggest that they were initiated the following year during the period of rapid growth.

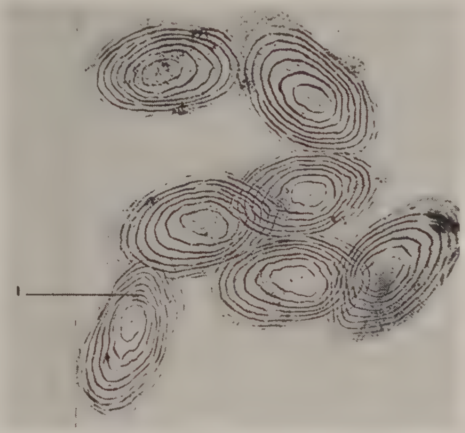

FIG. 6. Age-group II fingerling scales showing one normal and six retarded scales. 85 mm., Hatchery Creek, May 11, 1953.

Figure 7 represents a scale obtained from a small fish taken in the lake trap net. Annuli were formed at the end of the first, second, and third growing periods. A large scale increment was established during May and June of 1953. While the third annulus is incomplete on the left field, other scales from the same fish exhibit more typical third annuli.

In Figure 8 the compressed circuli in the central area of the scale are typical of those found in immature stream residents. The enlarged focus and the number of circuli within the first annulus indicate that it is a

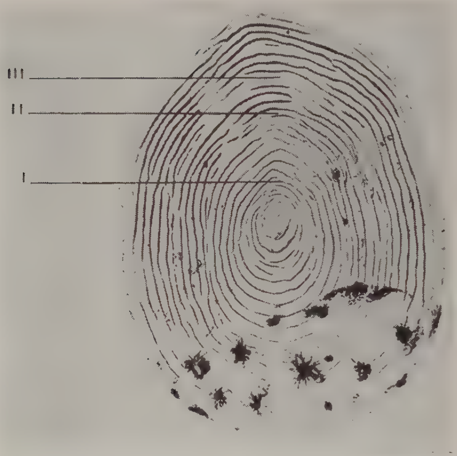

Fig. 7. Normal scale from Age-group III fish with three annuli. 280 mm., June 24, 1953, Lake trapnet.

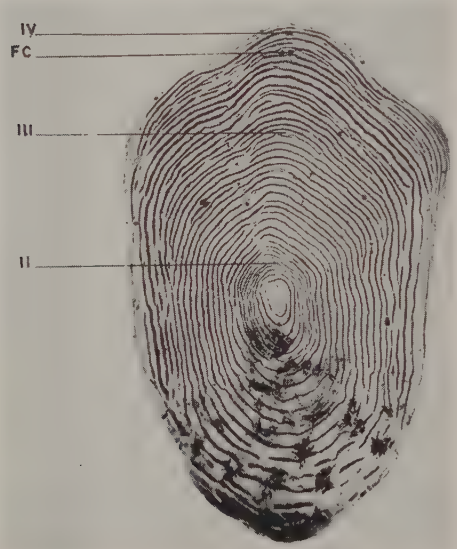

Fig. 8. Retarded scale from Age-group IV fish probably resident in a stream for two years. 305 mm., male, West Thumb, Aug. 4, 1953.

retarded scale. Movement into the lake apparently took place shortly after the resumption of spring growth in the third year. Rapid growth occurred during the following two years with a false check preceding the last annulus. Growth appears to have been slower during 1953, since only a small increment of scale growth is visible outside the last annulus.

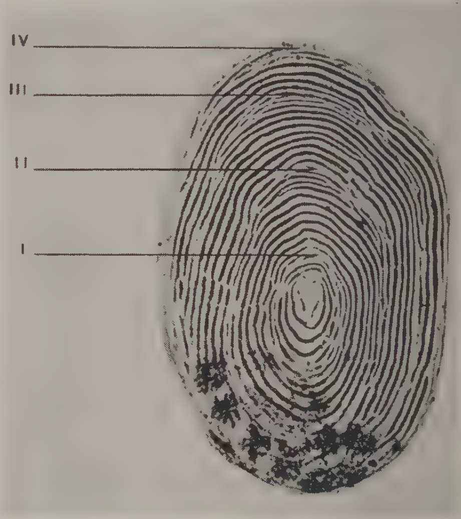

Fig. 9. Normal scale from Age-group IV fish. 310 mm., male, West Thumb, June 22, 1952.

Figure 9 represents a scale from a fish having moderate and uniform development. The annulus was completed shortly before capture. Near-maximum longevity is represented by the scale in Figure 10. No fish older than 7 years have been encountered in routine scale examination.

## FREQUENCY OF NORMAL AND RETARDED SCALES

Both adult and immature fish were studied to determine the frequency of normal and retarded initial annuli. Since first-year marks are rarely preceded by less than four circuli in Yellowstone Lake cutthroat trout, it is assumed that immature fish having three circuli or less during the first summer will usually not form annuli until they have completed their second summer. Approximately 31.5 per cent of the 1953 collection of fingerlings had scales with 4 or more circuli (Table 1) and these usually exhibited marginal growth retardation. However, about 67.3 per cent of all the fingerlings of Age-group I had at least some indication of scale

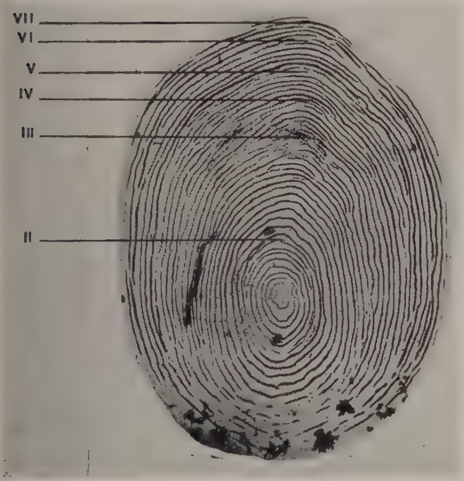

FIG. 10. Retarded scale from Age-group VII fish. 405 mm., male, West Thumb, June 15, 1950.

development. Specimens obtained from two small streams (Little Arnica and Little Thumb creeks) showed a greater than average incidence of scaleless fish. The fingerlings which were present in May were probably dominated by late-hatched individuals. A higher incidence of squamation occurred in collections from stream mouths and the lakeshore. Extent of scale development was positively correlated with the average size of specimens in the samples. The two spring-caught groups having the shortest average length had the smallest percentage of fingerlings with scale development to the four-circulus stage. Scales usually appeared first in fingerlings 41 to 44 millimeters in length. About one-third of the fingerlings collected demonstrated fall retardation of growth at the margin which is generally apparent in individuals only after they reach the four-circulus stage. It is of interest that about the same proportion of fingerlings and adult fish in the combined samples examined exhibited normal first annuli, *i.e.*, growth checks established during the first year of life.

Scales from 420 adult fish from West Thumb Dock and Fishing Bridge Dock were examined for first-year annuli. (The previously mentioned criterion of eight or more circuli within the first growth check was assumed to indicate failure to form the first year mark). About 40 per cent (unweighted mean) of both the West Thumb and Fishing Bridge samples displayed normal scales. The range of variation was from 30 to 60 per cent (Table 3).

Scale measurements and circulus counts were also made to permit quantitative comparisons of these values in normal and retarded scales. The circulus count included the ridges from the focus up to, and including, those in the first year mark. The anterior radius measurement was the mean value to the closest millimeter under a magnification of 110. The mean and standard deviations were calculated for each group of data. A '*t*' test was applied to determine the probability of a statistically significant difference between normal and retarded scales with reference to numbers

TABLE 3.—A COMPARISON OF NORMAL AND RETARDED SCALES WITH REFERENCE TO LENGTHS OF RADII AND NUMBERS OF CIRCULI WITHIN THE FIRST ANNULUS. SCALE SAMPLES WERE OBTAINED FROM CREEL SAMPLES ON WEST THUMB DOCK AND FISHING BRIDGE DOCK

| Area | Date | Number of scales | Percentage normal | Normal | | Retarded | |
|---|---|---|---|---|---|---|---|
| | | | | Mean number of circuli | Mean length[2] of radius | Mean number of circuli | Mean length of radius |
| West Thumb Dock | June 18–25, 1951 | 50 | 42 | 4.7 (0.71)[1] | 17.7 (4.24) | 8.3 (1.14) | 29.7 (4.57) |
| Fishing Bridge Dock | June 17, 1951 | 38 | 30 | 4.6 (0.68) | 16.4 (2.02) | 9.0 (1.73) | 29.6 (8.24) |
| West Thumb Dock | June 10–22, 1952 | 99 | 41 | 4.5 (0.88) | 16.2 (5.51) | 9.3 (1.94) | 31.1 (6.27) |
| Fishing Bridge Dock | June 12–23, 1952 | 96 | 56 | 4.9 (0.78) | 16.6 (4.08) | 8.7 (1.54) | 29.2 (6.06) |
| West Thumb Dock | June 16–July 14, 1953 | 59 | 35 | 4.3 (0.48) | 14.7 (3.39) | 8.9 (1.45) | 27.5 (5.46) |
| Fishing Bridge Dock | June 16–July 6, 1953 | 78 | 33 | 5.0 (0.82) | 17.1 (2.85) | 8.4 (0.82) | 28.0 (4.90) |

[1] Values in parentheses represent standard deviations.

[2] Lengths represent anterior scale radii measured in millimeters to the outside of the first distinct annulus and magnified by 110

of circuli and anterior radius measurements, respectively. In each case the differences were significant at the 99 per cent level.

In the course of the above scale examination, 10 scale mounts were observed to have both normal and retarded scales from the same fish. Evidently, annuli were established on a part of the scales on these fish (Fig. 6). Other scales on each of the specimens failed to form a year mark. Circulus counts and anterior radius measurements were made for both normal and retarded types. As it would be expected, the normal scales had smaller anterior radii (13.9 millimeters) with fewer circuli (4.1) than the average for normal scales in Table 3. Furthermore, the retarded scales had a greater average scale radius (31.6 millimeters) and more numerous circuli (9.4) than the average in retarded fish. This greater than average development of the retarded scales was to be expected since the presence of some normal scales on each specimen at the end of their first growing season suggests that the group were intermediate between the arbitrarily designated "partially" and "fully" scaled groups.

A further measurement was made to permit a comparison of anterior radius length at the end of two year's growth in normal and retarded fish, respectively. The average normal scale radius at second annulus was 34.2 millimeters—about eight per cent greater than in scales exhibiting retarded annulus development. The small difference in mean absolute scale size at the end of two years' growth would not necessarily bear on the relative usefulness of normal, as compared with retarded scales, in the back calculation of lengths at such annuli as are present on Yellowstone cutthroat trout scales.

The percentage of normally scaled fish appeared to be correlated with various factors in the life history. The influences of reproductive activities, seasons, age, and size, on occurrence of fish with normal scales were tested in an attempt to determine what possible quantitative relationships should be further investigated. Figure 11, which indicates the percentages of normal scales by 10-millimeter length intervals, shows that scales from more than 60 per cent of fish between 275 and 315 millimeters in length have normal first-year annuli. Fish 325 to 405 millimeters long were almost equally divided between normal and retarded types. The data on either side of the 320-millimeter mark were fitted with separate trend lines to show general tendencies.

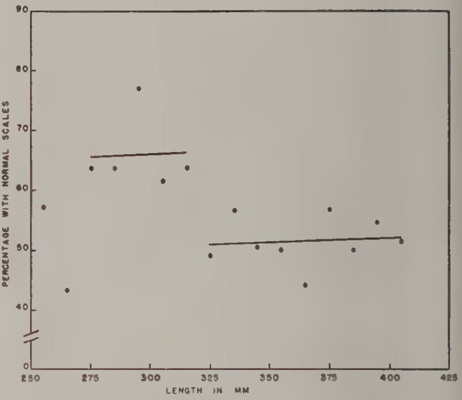

Fig. 11. Percentages of fish with normal scales plotted against total length. The sample includes 900 fish from the spawning and fishery runs during the 1950–53 seasons.

The points corresponding to lengths of 255 and 265 millimeters were omitted from the calculated line. The 265 millimeter length group represents a number of small Group III fish having a high percentage of retarded scales.

It is postulated that the greater proportion of fish with normal scales in the fish having average total lengths of 275 to 315 millimeters reflects differential recruitment. This hypothesis will be discussed later. A calculation of mean lengths of normal fish of Age-group III showed them to be about 10 millimeters longer than the comparable group exhibiting retarded annulus formation. Their larger size accompanied by possible developmental superiority appears to result in greater vulnerability of the normal trout. Nórmal fish predominate over retarded ones in the Age-group III spawners also (Fig. 12). The curves in Figure 12 show the frequency of occurrence of the two scale types in the early fishery (June) and the late fishery (August), each of the curves representing about 300 specimens collected in 1952 and 1953. West Thumb and Fishing Bridge areas are equally represented in the two curves.

The appearance of larger numbers of Group II fish in the late fishery distinguishes it from the early collection, in which only

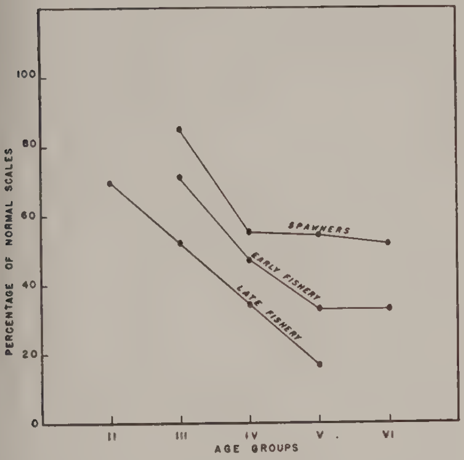

FIG. 12. Percentages of normal scales in various age groups of three seasonal samples from 1952 and 1953.

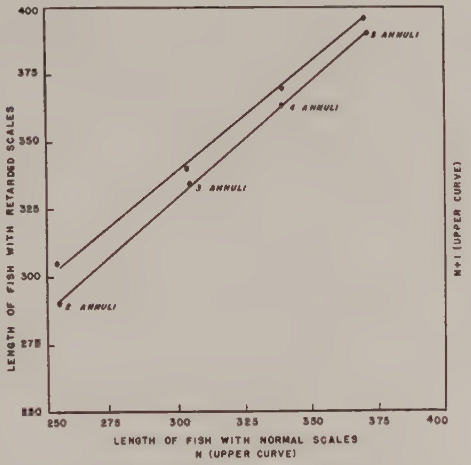

FIG. 13. Lower curve compares lengths of fish having normal scales with those having retarded scales. Points on curve represent lengths, in mm., at equal numbers of annuli. Upper curve shows lengths of normal fish at age $n$ plotted against length at $n+1$.

two of these showed up. Predominance of fish with first-year annuli in the group of youngest fish supports the hypothesis that unequal recruitment obtains in the population segment comprising Group II and Group III trout. The low percentage of fish with normal scales in the sample of Group V individuals from the fishery, is consistent with year-class data to be presented later.

In order to obtain additional comparative data on normal and retarded fish, their growth rates were compared according to the method described by Walford (1946). The present application is a modification of the original method since the values plotted against each other differ by something less than a yearly increment (Fig. 13). Walford's method consisted of representing the growth curve (or the portion of it above the inflection) as a straight line. The mechanics of plotting the data are quoted as follows:

"Using arithmetic graph paper, with body length represented along both the x axis and along the y axis, plot length at ages 1, 2, 3, 4, 5, . . . . $n$ on the x axis against length at ages 2, 3, 4, 5, 6, . . . . $n+1$ respectively on the y axis. . . . This line can be regarded as a sort of transformation of the usual growth curve. . . ."

The lower curve was obtained by plotting against each other the average lengths of fish with same numbers of visible annuli but with one group having normal and the other retarded scales, respectively. In other words, the length of normally scaled fish of an age group (x axis) was plotted against length of fish one year older having retarded scales (y axis). A straight line was fitted to the data by the least squares method. The equation was calculated to be: Mean length of normally scaled fish = −84 mm. + 1.163x mean length of retarded fish. The upper curve based on lengths of normal fish was included for reference. Average length at $n$ years on the x axis was plotted against length at $n + 1$ years on the y axis. The following equation describes the normally scaled fish: Length at $n$ years = −118.6 mm. + 1.234 × length at $n + 1$ years.

The vertical distance between the lines represents the mean length difference between fish having normal and retarded scales. The slope value suggests that in the sample of 900 fish, an appreciable degree of growth compensation occurs and the gap in respective lengths decreases each year. No correction was made to compensate for variability in length measurements resulting from the catch being spread over the whole season.

## ANALYSIS OF A SAMPLE AGE-FREQUENCY DISTRIBUTION

To illustrate the characteristics of seasonal creel samples, the age-frequency dis-

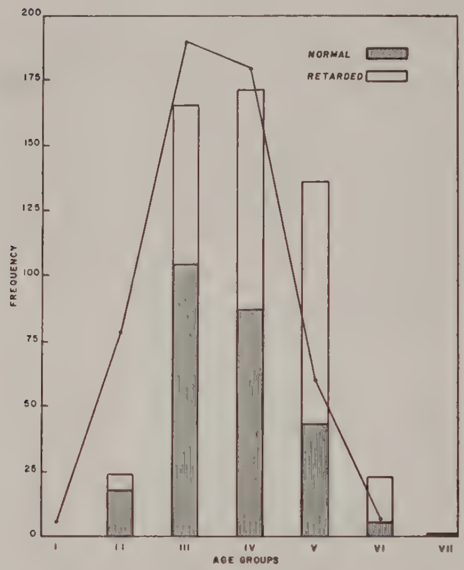

Fig. 14. Frequency distribution of normal and retarded fish in the weekly creel samples at Fishing Bridge Dock. The frequency polygon shows the distribution of the sample when aging was based solely on annulus counts irrespective of the hypothetical normal and retarded types.

tribution of the 1952 weekly collection from Fishing Bridge is shown in Figure 14. The histogram represents the frequency of normal and retarded fish in each age group. The frequency polygon shows the distribution when annulus counts are assumed to represent the correct age of the fish. It is evident that Age-groups III, IV, and V were the chief contributors to the 1952 collection. However, the composition of the catch changed considerably during the fishing season. For example, the Group III trout comprise only 16.5 per cent of the catch during the period up to the middle of July compared with 40 per cent for the remainder of the season. Apparently their vulnerability increased during the season and recruitment was nearly complete at the end of the summer. Fish of Age-group II formed a small part of the total catch but they were six times more numerous during the second half of the season.

During the first half of the season, the Group V fish comprised 32.7 per cent of the creel sample at Fishing Bridge Dock. The percentage declined to 18.8 per cent during the second half of the season. Undoubtedly the percentages were influenced by the location of the dock adjacent to a large area of spawning gravel at the lake outlet of Yellowstone River. A heavy take of gravid fish usually occurs in the early part of the season above and below the bridge and to a varying extent in other parts of the lake. The vulnerability of fish on the redds probably influenced the composition of the sample in June, and to a lesser extent in July. Although Figure 14 indicates that fish in their third and fourth years were incompletely vulnerable, the retarded segment of these age groups demonstrated greater delay in recruitment than did fish having normal scales. About 36.7 per cent of the sample of Age-group III fish had retarded scales whereas there were almost equal numbers of each type in fish a year older. The predominance of retarded fish in the group of five- and six-year-olds in the present sample is year-class phenomenon and will be discussed in connection with Figure 15.

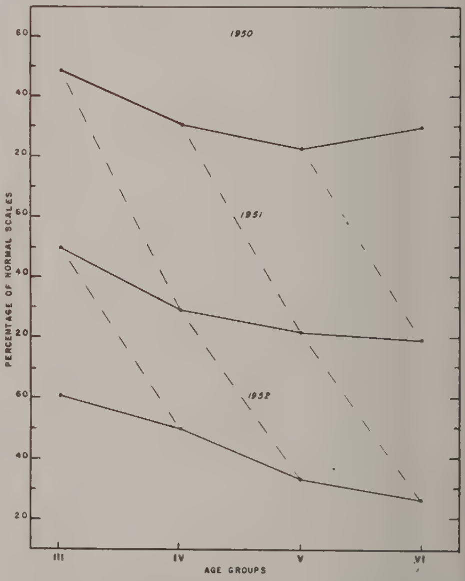

Fig. 15. Percentage frequency of normal scales of various age groups of black spotted trout collected at Fishing Bridge Dock, 1950–52. Broken lines connect points representing percentages of normal scales for individual year classes.

The cause and extent of the decline in percentage of normal types in progressively older fish was studied by analyzing data obtained from scale collections for 3 years at Fishing Bridge Dock. Between 400 and 500 scales comprised each year's sample which was drawn randomly from anglers using rowboats and those fishing from the wider ranging guideboats. The former are restricted to the vicinity of Fishing Bridge while the latter can move farther into the lake. The catch therefore represents fish from a radius of a mile or two from Fishing Bridge, with the bulk obtained from an area within one-half mile of the dock.

The data obtained from age determinations were arranged to permit comparison of the incidence of normal scales in each year class during consecutive years. The broken lines connect points representing percentages of normal scales in each of the four year classes for which age data were available. For example, the broken line beginning with Age-group III in 1951 shows that the percentage of normal scales for the 1948 year class was about 50 per cent and that the percentage of normal types was the same a year later. Agreement within the 1947 year class is not as good; the normal type comprised half the catch as three year olds, but made up only about 30 per cent of the samples as Group IV and Group V fish in 1951 and 1952, respectively. The percentage of normal types in the 1946 year class fluctuated between extremes of 30 and 21 per cent from the fourth to the sixth year. The 1945 year class was about 20 per cent normal during its fifth and sixth years. The available evidence suggests fairly good agreement in percentages of normal scales during successive years in each year class. Two factors may have caused the apparent decline in percentage of normal-type fish with increasing age when several years data were grouped. It appears that during some years, unequal recruitment of the normal and retarded segments of the fish population occurs in the fourth year of life. The 1952 catch data indicated that the bulk of the Group III fish were taken during the latter half of the season. While the early-season catch of this group was predominantly of the normal type, retarded fish constituted an increasing fraction of the catch as the season progressed.

In addition to differential recruitment, as discussed above, the ratio of normal to retarded types appears to be influenced by year-class differences. The preponderance of normal types in the Group III and Group IV fish in 1951 and 1952 collections (Figs. 12 and 15) is largely the result of the high percentage of normal fish in the 1948 and 1949 year classes. The older fish comprising the 1945 to 1947 year classes were predominantly retarded types. As a consequence of unequal recruitment and changing year class characteristics, the combined data showed a gradual decline in occurrence of normal fish from the younger to older age groups. It is anticipated that calculated ratios between normal and retarded fish will expedite routine aging of fish. Further study is needed to determine the applicability of these ratios for supplemental use in the recognition of discrete populations in the lake.

## SUMMARY

Fishery studies conducted on cutthroat trout in Yellowstone Lake have required investigation into the technique of age determination. Scales exhibit growth characteristics different from those of the same species in other waters at lower altitudes. The present studies have centered around the identification of scales on which the first annulus failed to form.

Cutthroat trout in Yellowstone Lake ascend spawning streams from May to late July. Fry emerge from late June to early September. Scale formation is initiated when the fish are 41 to 44 millimeters in length. Late-hatched fry may not attain this length before the interruption of growth in October, and thus may overwinter without scales or with only a few small scales. As a result, three developmental types of fry occur in the population: 1) fry overwintering in a fully-scaled state, 2) fry passing the winter partially scaled, and 3) fry overwintering without scales.

Annulus development after the first growing season occurred on the scales of fingerlings whose scale growth had reached the four-circulus stage. Late-hatched fry with rudimentary scales failed to establish a year mark during their first fall and winter period. These fish with "retarded" scales exhibit annulus formation following their second growing season. The term "retarded scale" is proposed to designate a scale that does not exhibit an annulus after the first growing

season, and the word "normal" is applied to a scale which did establish a year mark after one season's growth. Structural criteria have been established for the identification of the two types of scale development. The bases for differentiating the types are:

1. The numbers of circuli before the first annulus,
2. The size of the focus, or first circulus, and
3. The shape and density of the first one or two circuli.

Annulus formation begins before May 1 and is usually complete by the middle of June. Spawners appear to initiate scale growth before non-spawners.

Fingerlings taken late in the season and presumed to be in their first year of life included 35 per cent of fully scaled individuals. Those which were unscaled comprised 22 per cent, and those classified as partially scaled made up 42.7 per cent of the collections. Adults sampled from the 1951, 1952, and 1953 fisheries averaged 40 per cent normal.

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## Two Obscure Oyster Enemies in New England Waters

The American oyster, *Crassostrea virginica* (Gmelin), like many other pelecypod mollusks, is attacked by numerous enemies. Of these, the common starfish, *Asterias forbesi* (Desor), and two species of drills, *Urosalpinx cinerea* (Say) and *Eupleura caudata* (Say), are best known. Certain other enemies, perhaps because they are less striking in appearance, are comparatively small or, because they are not easily observed, are little studied and their activities are virtually unknown. This is true of the two forms which I have been recently observing in New England waters and which may be responsible for several "mysterious" mortalities of oysters, especially the young. One of these enemies is a flatworm and the other is a gastropod.

The worm is the polyclad, *Stylochus ellipticus* (Girard), identified by Libbie H. Hyman of the U.S. National Museum. It is usually found among oysters and old shells, on barnacles, and under rocks. Although the biology and destructiveness of the closely related species, *Stylochus inimicus* (Palombi), the so-called oyster "leech" of southern waters, have been extensively studied by several investigators, including Pearse and Wharton (*1*), very little is known of many aspects of the biology of *S. ellipticus* of New England waters, and the extent of its predation on our oysters has never been systematically observed or accurately estimated.

Our recent studies suggest that *S. ellipticus* plays a rather important role in the destruction of oysters, especially young ones. Laboratory observations have shown that the worms have little difficulty in entering and killing spat, even those measuring more than 2.5 in. in length. In one experiment, when ten worms were placed in the same dish with 30 healthy spat averaging about 1.7 cm in length, the worms killed 21 spat in less than a month. Not a single spat died in the control tray during that period. On at least two occasions, we saw the worms entering slightly open oysters. A day or two later, these oysters were gaping and soon died. A single worm was found in one oyster, and in the second, three worms were feeding on the oyster meat.

Since *S. ellipticus* has been found in comparatively large numbers in many areas where oysters grow, it, no doubt, can present an important problem. This contention is supported by Woelke's (*2*) finding that a closely related form, *Pseudostylochus* sp., causes extremely heavy mortality among three species of oysters grown on our Pacific Coast. Obviously, to gain the necessary information on the destructiveness of *S. ellipticus* and to devise methods for its control, more extensive studies of its life-history and ecological requirements are imperative.

The second enemy is a small gastropod, which was identified by Fritz Haas of the Chicago Natural History Museum as *Menestho* (*Menestho*) *bisuturalis* (Say). By some experts this gastropod is still placed within the genus *Odostomia*. It is a small, whitish snail that seldom reaches the size of 5.0 mm and is often found in shallow water. It belongs to the family Pyramidellidae. Fretter and Graham (*3*), who studied the snail's feeding apparatus in detail and also the mode of life of this group, report: "Pyramidellids are ectoparasites. Each species feeds on a particular species of host, usually a tubicolous polychaete or a lamellibranch mollusc, obtaining attachment to the body by means of the oral sucker, piercing the body wall with the buccal stylet and sucking blood, and perhaps tissue debris, by means of the buccal pump." These authors also gave a list of the hosts that have been found to be attacked by the members of the Pyramidellidae family. More recently, Cole and Hancock (*4*) added to this list *Odostomia eulimoides* (Hanley) and *Chrysallida obtusa* (Brown), which they found caused serious damage to the European oyster, *Ostrea edulis* L. Another pyramidellid was found by Medcof (*5*) to be attached to the siphons of *Mya arenaria* L. Medcof, however, thought that because it had no radula, it was commensal and not a predator.

Recently, I have been observing under laboratory conditions the behavior of *M. bisuturalis* in relation to young American oysters and found that it resembles the behavior described by Fretter and Graham for other Pyramidellidae. The typical feeding position of this snail is along the edges of the oyster shell, to which it attaches itself by the oral sucker. We have often seen groups of these snails occupying such a position on a single oyster. When the shells are open the snails protrude their proboscis to reach the soft parts of the oyster, usually the edge of the mantle. At first the oyster reacts to this stimulus by closing its shells but, apparently, it soon becomes accustomed to it and remains open, even if several *M. bisuturalis* are touching its mantle with their proboscis. In this respect the behavior of the oyster resembles, to a large extent, the reaction of other invertebrates attacked by related species of pyramidellids (*3*).

I found *M. bisuturalis* in large numbers on young oysters, especially those that came from shallow water areas. Although these snails may not be very successful in killing the oysters after the latter reach the size of about 1.0 cm, they, no doubt, interfere with the oysters' normal development and growth. This is often shown by the characteristically deformed shells of the young oysters that came from areas heavily infested with *M. bisuturalis*. The shells, instead of being flat, which is normal for shells of young oysters that are not too crowded, are deeply cupped and may have thickened edges. This abnormality is apparently due to injuries caused, by the activities of *M. bisuturalis*, to the edges of the oyster mantle, the organ that secretes the shell.

As in the case of *S. ellipticus*, it is apparent that we need more information on the feeding habits and general ecological requirements of *M. bisuturalis* before estimating its destructiveness and devising methods for its control.

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# ON UTILIZATION OF SALT WATER PONDS FOR SHELLFISH CULTURE

*By*

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# ON UTILIZATION OF SALT WATER PONDS FOR SHELLFISH CULTURE

During the last few years considerable interest has been displayed by the members of this laboratory and several of our colleagues in the possibilities of cultivating the American oyster, *Crassostrea virginica,* and other molluscs in small, enclosed or semi-enclosed salt water bodies, such as the numerous ponds that are found along the shoreline of a mainland and also on some large islands, such as Gardiners Island, New York; Martha's Vineyard, Massachusetts; and others. This interest is shown because the oyster beds in such large areas as Long Island Sound are severely damaged by frequent hurricanes and storms, and because the efficient control of oyster enemies there is extremely difficult. These disadvantages, however, can be eliminated if oysters are grown in relatively small bodies of water that are well protected against the action of storms, and where the control of oyster enemies is more feasible (Loosanoff 1954a).

Since several groups of workers are already either engaged or planning to engage in pond work, it seems desirable to offer a definite plan to evaluate the suitability of these salt water bodies for shellfish culture. For this, the following steps are suggested:

I. Make a general survey of the ponds to determine the following:

- a. Total area of the pond and its depth at different points.
- b. Character of the bottom, and extent of areas suitable for oyster beds.
- c. Presence and condition of native oysters in different parts of the pond.
- d. Occurrence and distribution of oyster enemies.

II. Conduct the following studies, the results of which should help to manage the ponds more efficiently:

- a. Changes in salinity, temperature, and pH of the water in different parts of the pond.
- b. Quantity of dissolved oxygen at different depths during different seasons of the year.
- c. Changes in quantities of nutritive elements in the sea water, such as phosphates, nitrates, *etc.,* and changes in plankton during the summer or, if possible, during the entire year.
- d. If the pond is semi-enclosed, determine its tidal range, and direction and velocity of tidal currents.
- e. If the pond has no connection with the outside, and its currents are sluggish, consider the possibility of installing current-producing and aerating devices.

In ponds that are connected with the outside by narrow channels, the possibility of controlling the tidal flow should be considered. For example, tide gates would help to retain the larvae until they are ready to set. Such gates, or other means of preventing the inflow of sea water, and maintaining the water level at a desired height, should also be valuable in controlling certain enemies. For instance, by allowing an inflow of fresh water and by blocking the entrance of sea water the salinity of the water in the ponds can be maintained for a sufficient time at a level low enough to kill drills, starfish and, perhaps, even crabs, without seriously affecting the oysters.

On the basis of the above observations tentative decisions may be made as to:

a. Location and extent of individual spawning beds.
b. Areas of bottom that can be utilized for cultch planting.
c. Quantity of shells that can be planted on each bed, and desirability of utilizing seed-collecting methods and devices other than the planting of loose shells.
d. Methods of control of oyster enemies to be instituted.

The peculiarities of the pond would help to decide what types of seed collectors should be used. These may be either loose shells, egg-crates dipped in cement, chicken wire bags filled with oyster shells (Prytherch 1930), or several others successfully used in Europe (Orton 1937) and Japan (Cahn 1950). Bags containing one to two bushels of shells could probably be successfully employed in most ponds. In others, however, where setting at the bottom will not take place because of unfavorable conditions, or because the enemies there are too numerous, other methods, such as use of rafts or floats supporting large quantities of cultch near the surface, as practiced by Japanese oyster culturists, could be advantageously employed.

In ponds having a channel connecting with the outside, the rafts carrying the cultch should be kept in a pond only long enough for the cultch to secure a sufficient number of spat. Under favorable conditions, this could be a matter of only a few hours. After that the rafts should be towed outside where the recently secured set can be planted, and the rafts, reloaded with cultch, returned to the ponds to obtain more set. By using this method, a large quantity of set may be procured from relatively small ponds in which a heavy setting continues throughout a long period.

III. For planting shells and for using other seed collectors at the most advantageous time, the following information would be desirable:

a Time of gametogenesis and beginning of spawning of oysters.
b. Relative intensity of spawning during different periods of the summer.
c. Rate of growth of larvae, length of larval period, and rate of survival of larvae to the setting stage. Staining larvae with vital dyes may help solve this problem (Loosanoff and Davis 1947).
d. Beginning, extent, and end of the setting period.
e. Daily intensity of setting in different areas of the pond.
f. Intensity of setting in relation to depth. This may be determined by using simple vertical collectors, which have proved adequate for such studies (Loosanoff and Engle 1940).

In shallow, warm ponds where the majority of adult oysters will discharge their spawn early in the summer, it may be advantageous, after the first wave of setting, to introduce another supply of ripe but unspawned oysters. Spawning of these oysters could be purposely delayed by keeping them in water of such low temperature that they could not discharge their sex products (Loosanoff and Davis 1951). By using this method, heavy spawnings in the pond could be induced at desired intervals throughout the summer.

IV. Observations should be made to determine the following:

a. Rates of growth and survival of spat on different beds of the pond from the beginning of setting to the beginning of hibernation.
b. Percent of survival of the set, if it is left in the pond throughout the winter. In some shallow ponds that freeze solid, the set may have to be removed to other grounds before the onset of winter.
c. Whether introduced spawners, if such are used, survive winters well and are able to develop spawn the following year. Compare their behavior and condition with those of native stock.

Selection of desirable races may be an important aspect in pond culture because many strains of oysters transplanted from one district to another do very poorly in new surroundings (Loosanoff and Nomejko 1951). Hence, it may be necessary, in some instances, to develop a sufficiently large stock of spawners from the pond population to assure enough set that will grow well under local conditions. On the other hand, some ponds may be populated with oysters which do well in these ponds, but which, upon transplanting to growing grounds in open waters where conditions are different, show unusually high mortality or poor growth. Obviously, such races are undesirable as spawn producers if the resulting set is not to be used locally. They should be removed or destroyed, and the ponds repopulated with a race that is known to do well in the area to which the set is to be transplanted. We can suggest two methods for destroying undesirable populations—first, lower the salinity in the pond for periods long enough to kill all the oysters, and second, use specific poisons.

If it is necessary to bring outside spawners into the ponds, it should be done early in the season, before the drills begin to deposit egg-cases. This will prevent carrying the egg-cases, together with the spawners, to the new locations. In some instances, it may be practical to obtain spawners from relatively brackish areas. Since these oysters will not be infested with starfish, drills, or sponge, they will not carry along these enemies.

The relative ease with which the ponds could be stocked with desirable strains of spawners suggests the possibility of developing races that have the qualities considered important in oysters. For example, in our waters it takes from four to six years to grow an oyster to marketable size. However, because of the recent advances in the cultivation of lamellibranch larvae, we can now try to breed out a race of fast-growing oysters which will reach marketable size in less than half that time. This desirable quality could be combined with others of equal importance, such as development of unusually large meats containing much glycogen. Obviously, if such races could be developed in the laboratory to be later used as spawners under natural conditions, the entire aspect of the northern oyster industry would become radically changed.

In conclusion, a few words should be said about fertilization of the ponds to offer more food to oyster larvae, spat, or adult oysters. This measure, which is so widely used in fresh water fish ponds (Neess 1949; Mortimer and Hickling 1954), has been considered, naturally, as one of the steps that will eventually assure success in the pond culture of oysters. Unfortunately, even now, sufficient information is not available as to the food requirements of oyster larvae or adults. We have learned, nevertheless, from recent experiments (Davis 1953) that oyster larvae are very selective in their food, and that usable food organisms are ordinarily not too numerous in the blooms which follow the addition of common fertilizers to sea water.

We have also found that green algae, such as Chlorella, which multiply rapidly after the addition of fertilizers, are not utilized at all by oyster larvae during their early stages of development (Davis 1950), and that heavy concentrations of Chlorella cells and their external metabolites will kill bivalve larvae (Loosanoff 1954b). Furthermore, it has been demonstrated that even adult oysters can feed efficiently only in relatively clear water, and that if the micro-organisms exceed certain numbers, they interfere with the feeding of the oysters and may eventually even kill them (Loosanoff and Engle 1947). Considering these dangerous aspects of fertilization, and also our exceedingly limited knowledge of food requirements of oyster larvae and adults, it may be advisable to proceed cautiously in fertilizing the ponds until more is known about this matter.

At present, we are principally interested in pond cultivation of the American oyster, *C. virginica.* Nevertheless, we think that similar approaches, with proper modifications, can be used for cultivation of other molluscs, such as clams, especially *Venus mercenaria,* and, perhaps, even scallops.

Consideration should also be given to the possibility of utilizing ponds for cultivation of the European oyster, *Ostrea edulis.* These oysters, which have been successfully grown in ponds and claires in Europe for some time (Orton 1937), were brought here from Holland in 1949 and planted in the waters of Connecticut and Maine (Loosanoff 1955). Because they survived, grew well, and propagated normally in their new environments, they should do well in many of our ponds.

This paper is largely based upon the outline which I prepared in March 1953, and later sent to my colleagues on the Atlantic and Pacific Coasts for criticism and suggestions. May I now offer my thanks to those who commented on it.

## SUMMARY

The article proposes the approaches for ascertaining the suitability of small, enclosed or semi-enclosed salt water bodies for the propagation of oysters and other molluscs. It suggests the steps to be taken in studying the physiological, chemical and biological characteristics of such ponds, and recommends certain devices and methods that should improve their conditions.

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70′ x 22′ x 6′ oyster dredger "Columbia", owned by Northern Oyster Co., Inc., Greenport, N. Y. The boat's capacity is 2500 bu. of oysters. She has a 330 hp. General Motors Diesel power plant with 4:1 Twin Disc reduction gear and 56 x 32 Columbian propeller. Surrette batteries and Gulf H.D. lubricating oil are used.

# Chemical Control of Shellfish Enemies Promising

*Oyster drills, crabs and flat worms among predators studied*

*by V. L. Loosanoff*, James E. Hanks and Anthony E. Ganaros*

WORK in developing methods for chemical control of shellfish enemies was initiated in 1948 at the Milford, Connecticut, fishery laboratory on a small scale, and was resumed on its present basis approximately two years ago. Hundreds of samples of various substances, mostly of organic nature, have been "screened" by Fish & Wildlife Service scientists to determine their effects upon molluscan enemies.

Some of these enemies are snails, usually called drills or borers, that drill holes through oyster shells and consume the meats. Starfish and crabs also directly attack oysters. Sponges establish on the shell and within a short time honeycomb throughout it. Some worms kill the oysters and eat their meats, and another group of worms lives in tubes formed within oyster shells, sometimes causing considerable damage. Several other groups of marine animals, such as sea grapes and slippery limpets, and sometimes mussels, although they are not direct enemies of oysters, often compete for food and space.

Thus far, several compounds have been found which show some promise toward the control of oyster drills and slippery limpets. Work in this direction is being continued. Several more promising chemicals have been found to control oyster worms and sea grapes. These chemicals may be used in dikes, such as those of the Olympia oyster industry, and in small ponds and claires, where necessary concentrations of the chemicals could be created to kill such important oyster enemies as the flat worm, or to control *Stylochus* of the Atlantic Coast, which kills thousands of bushels of young oysters annually.

Dissolved chemicals also can be used in controlling another worm, *Polydora,* that lives in the oyster shell, and the worm *Sabellaria,* that often smothers the oysters or scallops on their beds.

Solutions of the chemicals could be conveniently used on boats when transplanting oysters or cleaning the oyster beds. Under these conditions, dredgefuls of oysters could be dipped into vats containing such solutions and then, after a brief immersion, left on the deck for some time before planting. Such treatment might be sufficient in many instances to kill the worms.

### Methods of Using Chemicals

Several methods of using the chemicals have been devised, including the following:

1. Dissolved in water. This approach may be practical to kill oyster enemies in small, isolated bodies of water, such as salt water ponds, claires, dikes and large tanks, or for dipping into the solution oysters which are infested with enemies, such as sponges, worms, barnacles and, perhaps, some internal parasites.
2. Precipitated as a thin layer on the bottom over shellfish beds to repel enemies, such as drills, starfish, crabs, etc.
3. Combined with shell material of oysters, rendering it unsuitable for the existence of sponges, worms or other shell-dwelling organisms. This method even might discourage drills from drilling the shells of oysters.
4. Incorporated in food to be eaten by molluscan enemies, such as crabs, prawns, etc.

In testing the various chemicals on shellfish enemies, their effects also are observed on the behavior of oysters, clams, scallops and other useful animals. It is clear that if a chemical destroys an enemy but at the same time kills oysters or clams, it is of no value except, perhaps, in certain special instances of pond culture, where it might be desirable to exterminate an inferior local shellfish population to introduce a better race.

### Chemicals Kill Green and Horseshoe Crabs

It has been discovered that several chemical substances are extremely promising for the control of a large group of direct or indirect shellfish enemies, including green crabs, horseshoe crabs, prawns and shrimps. Some of the chemicals have been found to be effective in solution in sea water, others as food poisons, while several could be effectively used either way.

The chemicals have been utilized to combat eleven species within the phylum Arthropoda. These include the rock crab, blue crab, lady crab, mud crab, green crab, hermit crab, spider crab, common sand shrimp, mantis shrimp, copepods, and the horseshoe crab. The research showed that all these forms can be quickly paralyzed and killed.

The most striking results were achieved by using some of the chemicals provided by Chemagro Corporation, and used in preparation of such insecticides as Guthion and Dipterex. Results also have been achieved by incorporating in the bait other insecticides, such as the well known DDT, which was found several years ago to be extremely effective in control of barnacles and which, since then, has been widely used in oyster culture in Great Britain and other European countries.

(Continued on page 40)

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*Dr. Loosanoff is director of the Fish & Wildlife Service Laboratory at Milford, Conn., where the shellfish enemy studies are being conducted, and the Messrs. Hanks and Ganaros are associated with him.

# Crabbing Is Million-Dollar-Industry in Virginia

### By C. V. Forrest

THE blue crab has forged ahead in importance in the Virginia shellfish industry, until it now ranks second to the oyster. Virginia's yield of blue crabs in 1954 accounted for $1,638,000 of the State's $11,898,000 shellfish production. The crab pot is responsible for about two-thirds of the Virginia hard crab catch; with trot lines, scrapes, fykes, and dredges other types of gear used.

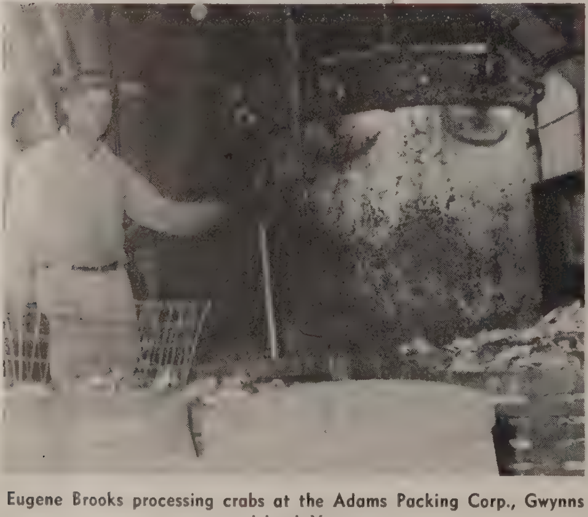
The early American Indian found that the supply of blue crabs was unpredictable, and ever since then, to some extent, the availability of the blue crab has fluctuated. Efforts toward management of the blue crab resource seem to have been based on the belief that fishing operations have been responsible for the periods of low abundance.

Following a poor crab season in 1940-41, Virginia established a sanctuary for sponge crabs near the mouth of the bay, where spawning females congregate. The catches increased sharply well into 1952. Then in 1953, the production in the Winter dredge fishery dropped off considerably.

Scientists believe that excessive protection of Virginia's blue crabs could result in an over-all loss to the seafood industry, with no advantage to future crab abundance. crabs should be caught before they die a natural death, making allowance for spawning escapement, according to the scientists. But it is not known as yet how many crabs should escape to spawn.

Much of the basic information required for crab conservation can be obtained by a comprehensive tagging program, an adequate system of catch records, and an annual measure of the success of spawning, according to [illegible] McHugh, director of the Virginia Fisheries Laboratory. Ill-advised legislation can adversely affect such a [illegible] management program. For example, Virginia had a law until July 1956 prohibiting one licensee from operating more than 50 crab pots. Such a law could not be enforced, so correct records of tagged crabs being caught were unobtainable. No one would admit having more than 50 pots.

The development by W. A. Van Engel of the Virginia Fisheries Laboratory of a tag that remains attached to the crab through shedding and the formation of a new shell, is a significant milestone in crab research. And skin diving, just put into practice by the Virginia Fisheries Laboratory, promises a revolution in eliminating guesswork about what is going on among the animals of the sea.



Eugene Brooks processing crabs at the Adams Packing Corp., Gwynns Island, Va.

Crabbers at New Point, Va., bringing their morning catches to sell to the 45' crab runner "Mary E.", which will take them to processing plants in Hampton. The "Mary E." is powered with a 185 hp. General Motors Diesel, and is skippered by Capt. Chase Morgan.

## How Crabs Are Marketed

A special industry is the marketing of the soft crab. Actually, very few soft crabs are caught, so crab houses and crab pounds are set up to handle peelers. These are found in the greatest abundance along the eastern shore of Virginia, and in the Piankatank, Rappahannock, and Potomac Rivers. The pounds in which the crabs are kept consist of a group of floats tied to stakes, with often a fence or breakwater.

Brought to the shedding house, the crabs are sorted according to their condition of shedding, and each group is placed in separate floats. During the peak of the season, a shedding house may handle as many as 40,000 crabs a week, and they must be examined every hour or so day and night to pick out the newly-moulted soft crabs. The crabs are graded and packed one against another in layers, iced ready for shipment.

Hard crabs, on the other hand, are prepared for market as cooked crab meat. Meat is graded as deluxe (lump or backfin), flake, and claw; packed in tinned steel cans, and iced for shipment. A few of the largest male crabs, referred to as "jimmies", are shipped alive direct to large cities for resale at seafood bars or as steamed hard crabs.

Shells are sold as containers for baked crab meat, or deviled crab, and the remainder of the scrap is dehydrated and ground for use in farm animal feed.

Virginia soft crabs and crab meat have a good reputation and a bright future. A Newport News firm, beginning with frozen "crabburgers" in 1946, has grown into a general frozen seafood industry, and is now producing from 10,000 to 12,000 packages of seafood daily. At present the company is enlarging its plant and doubling the number of workers.

## Life History of Crab

Hatching occurs in the Chesapeake Bay during the months of June, July, and August, when the crab is around two years old. Its life span is believed to be three years. A female produces approximately two million eggs, which are attached to her in a large yellow mass called the sponge.

*(Continued on page 41)*

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## Control of Shellfish Enemies

*(Continued from page 16)*

It is believed that within certain areas the chemical control of the stone crab now may become a possibility. Dr. R. W. Menzel, Florida State University, reports that the stone crab is a serious oyster enemy in the Gulf of Mexico, where it sometimes kills 25% of the oyster set.

With development of the new chemical methods, horseshoe crabs and several species of true crabs, including green and rock crabs, should pose no control problem in the near future in many areas. These crabs are great destroyers of soft and hard clams.

Complete chemical control of another crustacean, *Upogebia pugetensis*, which in its appearance resembles a crawfish, probably can be achieved soon without much effort. According to Dr. P. S. Galtsoff, this crustacean causes great difficulties to oyster farmers of Puget Sound by making passages under the oyster dikes, and also by piling up excavated sand and mud on oyster beds.

It is believed that predation by crabs in large, outdoor tanks, salt water ponds and certain natural bottoms used for propagation of shellfish, can be successfully controlled. Field and laboratory observations have shown that oysters, especially young ones, are frequently destroyed in New England waters by green, rock, mud and blue crabs.

Oyster crabs, which live inside oysters, are very prevalent in many areas. They cause damage to the gills of oysters and unfavorably affect quality of the oyster meats. This parasite probably can be eliminated in many cases by using solutions of chemicals.

Another enemy that lives inside mollusks is a parasitic copepod, which attacks Olympia and European oysters and other shellfish, including mussels. It is believed that if the infected oysters were allowed to pump water containing certain chemicals, the parasites would be killed.

Dr. H. Prytherch has shown that the mud crab serves as an intermediate host in the transmission of sporozoan, *Nematopsis*, which in many sections of the Atlantic and Gulf Coasts parasitizes the oyster. Chemicals could be used to exterminate these crabs in desired areas.

### Should not Endanger Commercial Shellfish

Another and extremely important point that always should be considered when control measures are applied under natural conditions, is the danger of killing desirable species. It is proposed to solve this problem by finding poisons that will kill only certain animals and leave others unharmed. Some promising results already have been obtained in this direction.

While evaluating chemicals to find the most promising ones to control crab-like animals, consideration is being given at the same time to a way in which these substances can be used on natural and artificial shellfish grounds. At present, cooperation is under way with commercial enterprise to incorporate the poisons and the substances that should attract the enemies in special pellets which can be mechanically distributed over the grounds.

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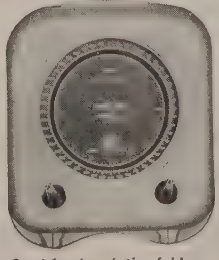
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## New Jersey Fishermen Want To Fish Closer to Shore

A proposal that would permit dragging for fish closer to the shore line was presented last month to the New Jersey Fish & Game Council by William C. Lundsford, Wildwood Crest. According to his proposal, a season for whiting and ling fish would be set from December 1 to March 31. At present, fishermen may drag all Winter, but must remain two miles from shore.

Since commercial fishermen make their living by fishing, it is felt that they should be permitted to get closer to shore, especially at this time of year, when they would not be interfering with resort attractions. The proposal has been approved by members of the Commercial Fishermen, Trappers and Land Owners Assoc.

## Would Make Mechanical Clam Tongs Illegal

A resolution making illegal the use of clamming tongs operated by mechanical means was approved last month by the State Shell Fisheries Council. The resolution will be sent to the State Attorney General's office. If approved, it would become an amendment to a present law governing the use of such tongs.

The Council agreed to study and make recommendations on the dredging of crabs along the State's coastline. It has been reported that many young crabs are dredged up in the early Spring before they grow to useful size.

## Landings Set New Record

Landings of fish and shellfish at New Jersey ports during August amounted to 116.5 million pounds valued at 2.2 million dollars. This production was 56.6 million pounds above reported landings for the same period last year, due primarily to excellent weather and an abundance of seasonal varieties of fishery products. August landings this year were the largest in any one month since publication of such figures began.

Almost all of the bluefish were taken by hand lines. These fish generally ran smaller than usual, and prices paid to fishermen dropped accordingly. The catch taken by otter trawls consisted principally of fluke, sea trout and scup, practically all landings being made by inshore craft.

## South Carolina Trawlers Shrimping off Florida

Charleston trawler operators have begun their annual pursuit of shrimp in the warmer waters of the Gulf of Mexico and Florida. Better size shrimp are usually found in tropical waters after the last of October and November. The legal shrimp season in South Carolina does not close until December 15.

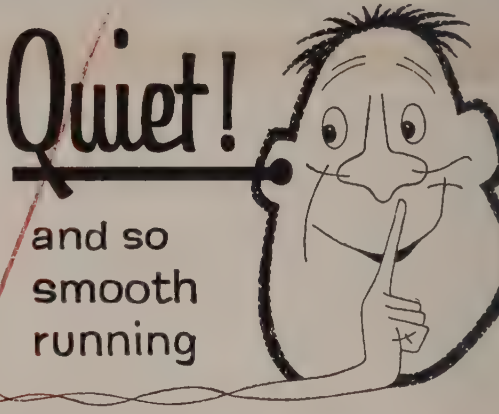
The *Miss Dona, Silver Star, Barbara Lee, Miss Cain Hoy* and the *Carol El* are already in the South. Most shrimp boats are 60 ft. or over, and will stay South for the Winter.

It is estimated that from 12 to 15 boats from the Mt. Pleasant area will be operating in Florida by the middle of this month. They will return to Charleston about March or April of next year.

## Boring Sponges Damage Oysters

According to Dr. Sewell Hopkins of Texas A & M, boring sponges in the coastal waters of South Carolina are having a destructive effect on the State's oyster crop. Dr. Hopkins has written a paper in which he describes the tiny perforations made in the shells of oysters by the boring sponges, and the weakening effect they have on the oysters.

He also told of the influence dry weather can have on oysters, and said that salt-loving marine organisms have invaded coastal waters due to a 22.5 deficiency in rainfall over the past two years.



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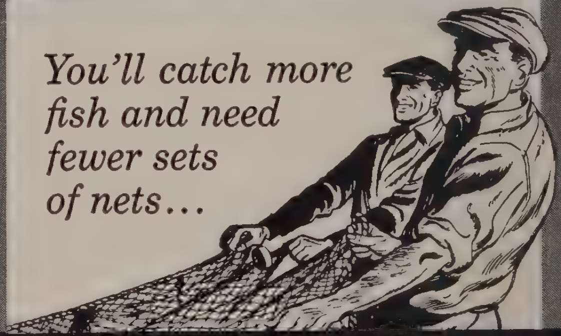
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# RELATIVE INTENSITY OF OYSTER SETTING IN DIFFERENT YEARS IN THE SAME AREAS OF LONG ISLAND SOUND

V. L. LOOSANOFF AND C. A. NOMEJKO

*U. S. Fish and Wildlife Service, Milford, Conn.*

Quantitative studies of marine bottom invertebrates have been conducted since the early part of the century, and the results have substantially enriched our knowledge and understanding of aquatic communities. The contributions of many workers to this important branch of marine biology have been reviewed by several authors, including Spärck (1935) and, more recently, Sanders (1956).

Regardless of the progress made, there still remains one aspect of this field which has been relatively neglected but which should be of special interest to many students of bottom communities. In general, it concerns the recruitment of the new year-classes of such forms as mollusks and echinoderms that have pelagic larvae which, after a free-swimming period, descend to the bottom and metamorphose, the act commonly called setting. In particular, it deals with the variations in the intensity of setting of the same species in the same area in different years, and comparing these variations with those of other, nearby areas.

Our long-term studies of the biological events of Long Island Sound give us the opportunity to discuss certain aspects of this problem in relation to the American oyster, *Crassostrea virginica.* The conclusions are based on data collected during the past 12 years, 1944 through 1955, from ten chosen areas. The locations of these areas, which we shall call stations, are shown in Figure 1. They were confined to three depths— 10, 20 and 30 feet—and represented three major oyster-producing sections of Long Island Sound, namely, New Haven, Milford and Bridgeport. The combined area of these sections is approximately 80 square miles.

The intensity of setting at each of the stations was evaluated by counting the number of recently set oysters on special collectors consisting of wire mesh bags filled with old oyster shells (Prytherch, 1930). This is the standard method in use at our laboratory for over 20 years, and with which most oyster biologists and oystermen are now well familiar (Loosanoff and Engle, 1940; Loosanoff, Engle and Nomejko, 1955). It is important to emphasize that the locations of the stations remained the same during the 12 years, and that the methods of determining the intensity of setting were identical for all stations.

To evaluate the relative productivity of each station, we employed a simple ranking method by giving, each year, Rank 1 to the most productive station, Rank 2 to the next most productive, and so on, until the least productive was given Rank 10. For example, for 1944, Station 1, the most productive, was given Rank 1; Station 2, the least productive, Rank 10; Station 3, Rank 9; etc. (Table I).

To determine the relative productivity of the stations during the entire 12-year period, we expressed the rank of each station as the sum of its yearly ranks (Table I). Naturally, the stations that were generally better producers and, therefore, entitled to low ranks, such as Station 9, showed lower sums than the stations that were less productive. On the basis of this total score, we gave a long-range rank to each station.

The question immediately arose as to whether these ranks would remain approximately the same if the stations were graded for their performance only during the years of better sets, namely, 1944, 1945, 1946, 1953 and 1955. However, our analysis showed that the ranks of the stations for these years were not substantially different from the long-range ranks based on the 12-year observation period (Table I).

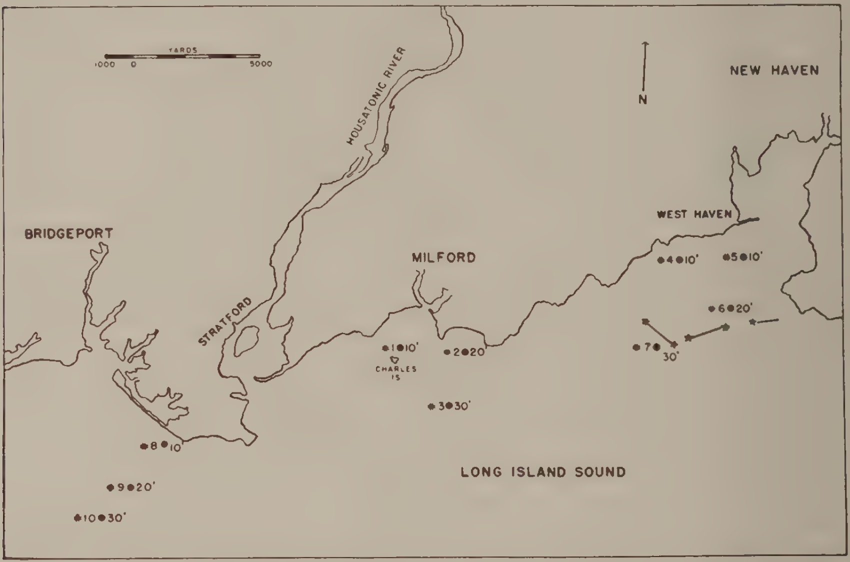

Figure 1. Locations and depths (in feet) of ten stations established for observation of oyster setting in Long Island Sound, 1944–1955.

A close study of the data provided information as to the relative importance of the stations, the depths, and the areas in the different years. It was established that, excluding Stations 2, 3 and 4, each station ranked first at least one year out of 12. It was also found that a 10-foot station ranked first, five times; a 20-foot station ranked first, three times; and a 30-foot station, four times. Thus, considering that at the 10-foot depth we have one station more than at 20 or 30, it appears that Rank 1 was occupied by stations of each of the three depths the same number of times.

If we add the sums of the yearly ranks of all the stations at the same depth and then calculate the average sum of the yearly ranks of these depth-groups, we will find that the 10-foot stations have a score of 65.2; 20-foot stations, 57.3; and 30-foot stations, 75.7. Thus, the 20-foot stations seem to be generally somewhat more productive than the others, while the 30-foot stations appear to be the least productive of the groups at all three depths. The latter conclusion, however, may not be well founded because the low rank of the 30-foot stations is chiefly due to the history of setting at Station 3 which, through the 12 years, was consistently one of the poorest, never rising above sixth place; whereas another 30-foot station, Number 10, was, in more than half the instances, among the five best stations, and ranked first on three occasions (Table I).

TABLE I

*Rank order of the sampling stations representing oyster-setting areas of Long Island Sound during the 12-year period, 1944–1955*

| Areas | Milford | | | New Haven | | | | Bridgeport | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Stations | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| Depth in feet | 10 | 20 | 30 | 10 | 10 | 20 | 30 | 10 | 20 | 30 |
| Years | | | | | | | | | | |
| 1944 | 1 | 10 | 9 | 5 | 2 | 4 | 7 | 6 | 8 | 3 |
| 1945 | 1 | 4 | 8 | 10 | 6 | 5 | 9 | 2 | 3 | 7 |
| 1946 | 6 | 9 | 8 | 10 | 5 | 1 | 3 | 7 | 2 | 4 |
| 1947 | 7 | 6 | 8 | 9 | 5 | 2 | 10 | 1 | 3 | 4 |
| 1948 | 8 | 6.5* | 10 | 3 | 6.5* | 5 | 2 | 4 | 1 | 9 |
| 1949 | 8 | 4 | 9 | 3 | 5 | 7 | 1 | 2 | 6 | 10 |
| 1950 | 7 | 9 | 10 | 8 | 4 | 6 | 2 | 5 | 1 | 3 |
| 1951 | 9 | 10 | 7 | 8 | 4 | 6 | 5 | 3 | 2 | 1 |
| 1952 | 1 | 5 | 10 | 7 | 4 | 6 | 8 | 3 | 2 | 9 |
| 1953 | 7 | 3 | 8 | 10 | 4.5* | 6 | 9 | 4.5* | 2 | 1 |
| 1954 | 7 | 2.5* | 9.5** | 9.5** | 1 | 5 | 6 | 8 | 4 | 2.5* |
| 1955 | 4 | 5 | 6 | 10 | 7 | 9 | 8 | 3 | 2 | 1 |
| Sum of 12 yearly ranks | 66 | 74 | 102.5 | 92.5 | 54 | 62 | 70 | 48.5 | 36 | 54.5 |
| Long-range rank, 12 years | 6 | 8 | 10 | 9 | 3 | 5 | 7 | 2 | 1 | 4 |
| Rank for 1944, 1945, 1946, 1953, 1955 | 3 | 7 | 9 | 10 | 5 | 6 | 8 | 4 | 2 | 1 |

* ** Indicate a tie between stations for the same rank.

The data were also used to evaluate the relative productivity of the three areas under observation, namely, New Haven, Milford and Bridgeport (Fig. 1). By adding the ranks of all the stations, as given in Table I, for each area and year, and dividing the resulting figure by the number of stations, the average station rank for each area was determined (Table II). Accordingly, each area was given a yearly rank, the one with the lowest score occupying the first or most productive position. The sums of the average yearly ranks for each year, as shown at the bottom of Table II, were also determined. It was found that the Bridgeport area occupied first rank, or the best producing position, nine years out of 12, and never held third or last place. The New Haven area was next, occupying first rank for three years. The Milford area, however, never reached the highest position, ranked second only four times, and was in the third, or lowest position for the remaining eight years.

We cannot offer a fully satisfactory explanation for the variations or, in some instances, stability from year to year in the relative productivity of our stations. Such considerations as original number of eggs discharged; mortality of larvae due to diseases, enemies, or lack of food; and several others are, of course, of importance. Nevertheless, there is little doubt that the intensity of setting of oysters at all stations depends to a large extent upon the peculiarities of the inshore system of water currents.

TABLE II

*Average yearly station-ranks of the New Haven, Milford and Bridgeport areas, and general rank of each of these three areas for each year of 1944–1955 period. Sums of average yearly ranks of stations of each area, and the ranking of the areas during the entire 12-year period are also given*

| Years | Areas | | | Ranks | | |
|---|---|---|---|---|---|---|
| | New Haven | Milford | Bridgeport | 1 | 2 | 3 |
| 1944 | 4.50 | 6.67 | 5.67 | New Haven | Bridgeport | Milford |
| 1945 | 7.50 | 4.33 | 4.00 | Bridgeport | Milford | New Haven |
| 1946 | 4.75 | 7.67 | 4.33 | Bridgeport | New Haven | Milford |
| 1947 | 6.50 | 7.00 | 2.67 | Bridgeport | New Haven | Milford |
| 1948 | 4.13 | 8.17 | 4.67 | New Haven | Bridgeport | Milford |
| 1949 | 4.00 | 7.00 | 6.00 | New Haven | Bridgeport | Milford |
| 1950 | 5.00 | 8.67 | 3.00 | Bridgeport | New Haven | Milford |
| 1951 | 5.75 | 8.67 | 2.00 | Bridgeport | New Haven | Milford |
| 1952 | 6.25 | 5.33 | 4.67 | Bridgeport | Milford | New Haven |
| 1953 | 7.38 | 6.00 | 2.50 | Bridgeport | Milford | New Haven |
| 1954 | 5.38 | 6.33 | 4.83 | Bridgeport | New Haven | Milford |
| 1955 | 8.50 | 5.00 | 2.00 | Bridgeport | Milford | New Haven |
| Sum of average yearly ranks | 69.64 | 80.84 | 46.34 | Bpt.—9 yrs.<br>N. H.—3 yrs.<br>Mfd.—0 yrs. | N. H.—5 yrs.<br>Mfd.—4 yrs.<br>Bpt.—3 yrs. | Mfd.—8 yrs.<br>N. H.—4 yrs.<br>Bpt.—0 yrs. |

The complexity and characteristics of these currents in the oyster-producing section of Long Island Sound are still relatively unknown because no detailed study has ever been made. We know, however, that planktotrophic larvae, with comparatively longer pelagic lives, like those of oysters and many other pelecypods, are carried by water masses and that their distribution is controlled by the currents. Under certain conditions the direction of the currents may be so changed that the larvae will be carried away from the areas where setting normally takes place, and eventually perish. In other instances, as reported by Coe (1953) for *Donax gouldi*, an enormous increase in the population of a species may occur because swarms of pelagic larvae, about ready to set, are unexpectedly brought inshore by water currents. Hence, it is understandable that the productivity of small areas, such as those designated for our stations, should, in general, be more affected by minor changes in larvae-carrying currents than that of larger areas, such as New Haven, Milford or Bridgeport, which cover many square miles of oyster-producing bottom, and should certainly display more stability in maintaining their relative positions.

These observations emphasize the importance of studying the different aspects of local minor currents, including their direction, velocity and stratifications. They also indicate the importance of understanding the relationship between the behavior of such currents and the locations of the spawning beds, of oysters or other mollusks, where the larvae originate.

Our studies suggest, moreover, that minor currents are often extremely precise in their behavior. This was well demonstrated by observations on the intensity of setting of oysters at our Station 10, located several miles from the shore and in comparatively deep water, but where, nevertheless, heavy setting continued steadily day after day for as long as three or four weeks because the currents consistently brought a supply of ready-to-set larvae to that point. In 1955, this regularity was not noticeably affected even by the strong winds of hurricane "Connie" nor by the winds and record-breaking floods of "Diane." Finally, they imply the potential danger of interfering with established combinations of the favorable ecological conditions existing on the bottom by modifying its contour so as to change the directions of the local currents. Although these changes may not affect commonly studied factors, such as temperature and salinity, they may, nevertheless, so alter the currents that the larvae will be carried to new areas, some of which may not be suitable for their setting.

We wish to express our appreciation to Barbara J. Myers for her assistance in analyzing these data, and to our colleagues, Harry C. Davis and Rita S. Riccio, for their helpful suggestions in preparing this paper.

## SUMMARY

1. During the 12 years of observations none of the stations, representing relatively small bottom areas, always occupied a position among the best oyster set producers.

2. If larger areas instead of individual stations were compared, a definite tendency of the Bridgeport area to be more productive than the others was evident.

3. There was no evidence that the stations located at a definite depth, such as 10, 20 or 30 feet, consistently produced better sets of oysters than the stations at other depths.

4. There may be a great variability in the density of oyster set even within a given depth and district in the same year. For example, Stations 4 and 5, although located in the same district and at the same depth, showed a rather different standing with Long-Range Ranks of 9 and 3, respectively.

5. Local minor water currents are important in the relative productivity of bottom areas.

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JOHN C. MARR

# THE "CRITICAL PERIOD" IN THE EARLY LIFE HISTORY OF MARINE FISHES

EXTRAIT DU JOURNAL DU CONSEIL INTERNATIONAL
POUR L'EXPLORATION DE LA MER
VOL. XXI, No. 2. 1956

# The “Critical Period” in the Early Life History of Marine Fishes.

By

**John C. Marr**[1],
U. S. Fish and Wildlife Service, La Jolla, California.

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## Introduction.

Postulation of the existence of a "critical period" (of high mortality rate) during the early life history of those marine fishes which are extremely fecund was ably dwelt upon forty years ago by Hjort (1914). This idea has been rather generally accepted, if not as fact, at least as a very reasonable hypothesis; it has had a profound effect upon marine fishery biology.

The implications of this hypothesis are rather far-reaching and it is therefore pertinent to briefly review the hypothesis, to consider the form that early survival curves may take, and to contemplate the accumulated evidence bearing on whether or not there is in fact such a "critical period" *in nature*.

### The hypothesis.

Hjort (1914: 203) stated: "The rich year classes [of fishes] thus appear to make their presence felt when still quite young; in other words, the numerical value of a year class is apparently stated at a very early age, and continues in approximately the same relation to that of other year classes throughout the life of the individuals." And (p. 204): "We must therefore look to the later stages of the eggs to find the conditions which determine the numbers of individuals in any year class. This again leads us to the question, at which stage of development the most critical period is to be sought. Nothing is known with certainty as to this; such data as are available, however, appear to indicate the very earliest larval and young fry stages as most important."

He also suggested two possible methods by which such early reduction in numbers might be accomplished, and subsequently (Hjort, 1926: 33) re-stated these as:—

"1. That those individuals which at the very moment of their being hatched did not succeed in finding the very special food they wanted would die from hunger. That in other words the origin of a rich year-class would require the contemporary hatching of the eggs and the development of the special sorts of plants or nauplii which the newly hatched larva needed for its nourishment.

2. That the young larvae might be carried far away out over the great depths of the Norwegian Sea, where they would not be able to return and reach the bottom on the Continental shelf before the plankton in the waters died out during the autumn months of their first year of life."

Following Hjort's hypothesis, and in spite of a notable paucity of observational or experimental data, fishery biologists have come to accept it as almost axiomatic that year-class size of marine fishes is determined during the first few days or, at most, months, of life. (There is, however, a large group of fishery biologists who assume, again in spite of a lack of data, that year-class size is determined even before the eggs are spawned; i.e., that it is a function of stock size. This assumption does not require that there be or be not a critical period. But it does require that the net survival rate either be constant in all years or that it be some function of stock size, so that the number of eggs spawned determines the size of the resulting year-class.)

At least three different life-history stages have been suggested as the one which is most critical. Rollefsen (1930) has suggested that cod (*Gadus callarias*) year-class size is determined in the egg stage, according to whether or not the eggs are damaged by wave action. Hjort (1926) has, as already noted, suggested that the time of yolk absorption is critical, success or failure of the year-class depending on an abundant food supply when the larvae commence feeding. Hjort (1926) and Sund (1924) have suggested that the larval stages may be most critical, depending on the influence of the currents on the distribution of the larvae to favourable or unfavourable locations.

Even earlier, Fabre-Domergue and Biétrix (1897), who were interested primarily in marine fish culture, had observed high larval mortality rates in rearing trials occurring at about the time of the completion of yolk absorption. To this phenomenon they applied, for the first time, the term "la période critique". Since that time references to this critical period are common in the works of marine fish culturists and, more recently, in the works of those attempting to rear marine fishes for experimental purposes (see, for example, Morris, MS).

It is obvious, as Hjort reasoned, that in species which spawn large numbers of eggs, there must be a tremendous mortality sometime between the time the eggs are spawned and the time at which the fish assume the adult form and habit. For example, the size of the spawning

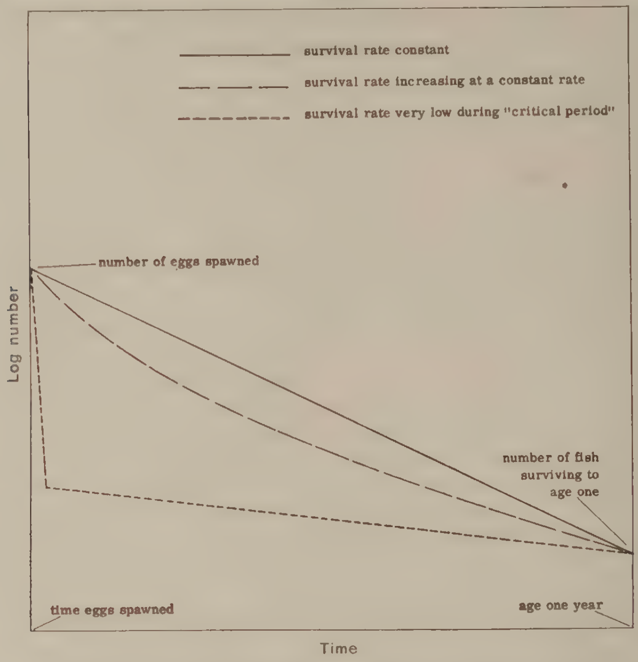

Figure 1. Three forms (schematic) which fish survival curves could take, from the time the eggs are spawned to age one year.

stock of the Pacific sardine (*Sardinops caerulea*) in 1942 was $7{\cdot}6 \times 10^9$ fish (this is a minimal estimate, based on fish actually caught, and does not take into account deaths from natural mortality; see Clark and Marr, 1955). According to Clark (1934) each female sardine (of the length common in the population then) would have spawned, on the average, about 100,000 eggs per year. The 1942 stock must therefore have spawned about $3{\cdot}8 \times 10^{14}$ eggs. A minimal estimate of the 1942-class, which resulted from these eggs, is $2{\cdot}3 \times 10^9$ fish at age two years, or a reduction to 0·000605 $^0/_0$ of the original number in two years' time. Similar examples may be found in all species for which the appropriate data are available.

**Form of early survival curves.**

While the reduction in population numbers from eggs to, say, age one year is obvious, it is difficult and costly to make observations on the nature of this reduction and the time at which it occurs. As a consequence, data on early mortality or survival rates in the sea have been collected for only a few species. Before considering these data, it is pertinent to consider the form that early survival curves might be expected to take.

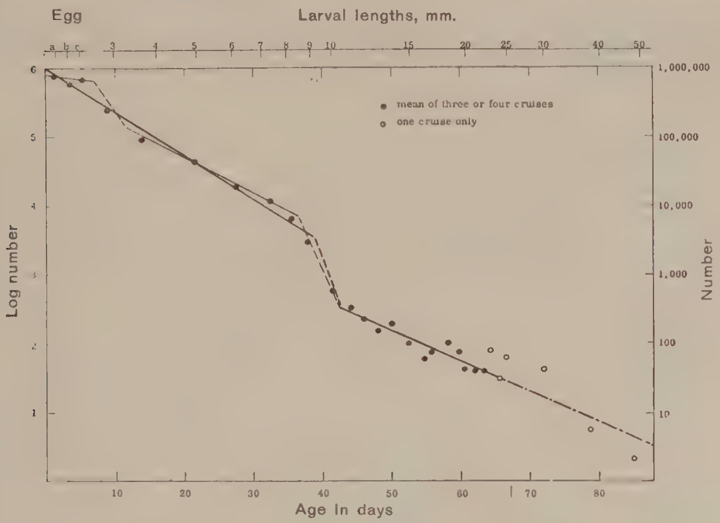

Figure 2. Survival of young stages of Atlantic mackerel. (From Sette, 1943, Figure 17.)

Three such hypothetical survival curves are shown in Figure 1, in which the logarithms of the numbers of fish are plotted as a function of time. The curves start with the number of eggs spawned and represent three different types of survival to age one year. The number of eggs spawned and the numbers of fish surviving to age one year are the same in each example.

The "critical period" type of survival curve is shown by the dashed line. This curve is characterized by an extremely low survival rate (or high mortality rate) during the early "critical period". In the hypothetical example given, a constant survival rate is shown following the "critical period", but this portion of the curve could, of course, be of some other form. In survival of this type, it is usually hypothesized that the extent of mortality during the "critical period" is the dominant factor in determining the numbers surviving to age one.

A second type of survival curve, in which the survival rate is increasing at a constant rate, is shown by the broken line. Even though, as in the "critical period" curve, mortality rate is greatest at the earlier stages, it is the survival rate over the whole period which determines the numbers surviving to age one.

The third hypothetical curve, shown by the solid line, represents a constant survival rate over the entire period. In this type of survival the numbers surviving to age one are, obviously, determined by the constant survival rate.

All of these curves are idealized beyond what one would expect to observe in nature. Furthermore, it would be possible for survival to follow some combination of these hypothetical curves, or some completely different curve. For example, the survival rate might decrease at a constant rate, although this does not seem likely.

**The evidence.**

The first work that I have been able to find which deals critically with the early survival rate of marine fish is that of Sette (1943) on the Atlantic mackerel (*Scomber scombrus*) of North America. He gives a survival curve of the 1932-class over the first 85 days of life, during which period the larvae attain a length of about 50 mm. His Figure 17 is reproduced here as my Figure 2. A thorough discussion is given by Sette (1943: 191—208) of the derivation, reliability, possible sources of error, and significance of this survival curve.

Pertinent to this discussion is the slope of the curve. Of the two possible interpretations of the data given by Sette, both are characterized by a period of high mortality at age 40 days or larval length of 9—10 mm. One interpretation involves a uniform survival rate up to age 40 days, the alternative involves three different rates during this period. In neither case is the mortality rate such that it could be considered to constitute a "critical period" at the time of yolk absorption. Indeed, as Sette (1943: 203) stated: "If any one period is to be singled out as the most critical, it must be the ensuing period during the transition from larval to post-larval stages, when in passing from 9 to 11 mm., the numbers are reduced by 90 percent in the short space of about 3 days." And (1943: 204) "... one may say that about one-third of the mortality was suffered during the so-called 'critical' stages, and the other two-thirds during what might be called 'non-critical' stages."

The change in the estimated survival curve at larval size 9—10 mm. may arise from one of three causes; (1) it may actually represent a period of high mortality, (2) it may represent a period when the larvae are not caught in proportion to their true abundance (either because of increased swimming and net-dodging ability or because some of them have moved out, horizontally or vertically, of the water sampled by the nets), or (3) it may represent a temporary period of reduced rate of growth. Some evidence for increased dodging ability is found in the fact that at larval size 9—10 mm. great changes occur in the fins. In fact, Sette (1943: 171) characterizes this as the "transition phase" and states that "... the tail fin makes its greatest changes, the second dorsal fin and finlets and the anal fin and finlets are all developed within this size range ..."

However, he concluded that larval sizes up to 22 mm. were sampled in proportion to their true abundance, on the basis of catches of 1- and 2-m. nets hauled at the same stations. That is, the 1-m. net took larvae in about the same numbers as did the 2-m. net, per volume of water strained. This would not necessarily rule out the possibility of dodging, however, if the maximum size net which the larvae were able to dodge was greater than 2 m.

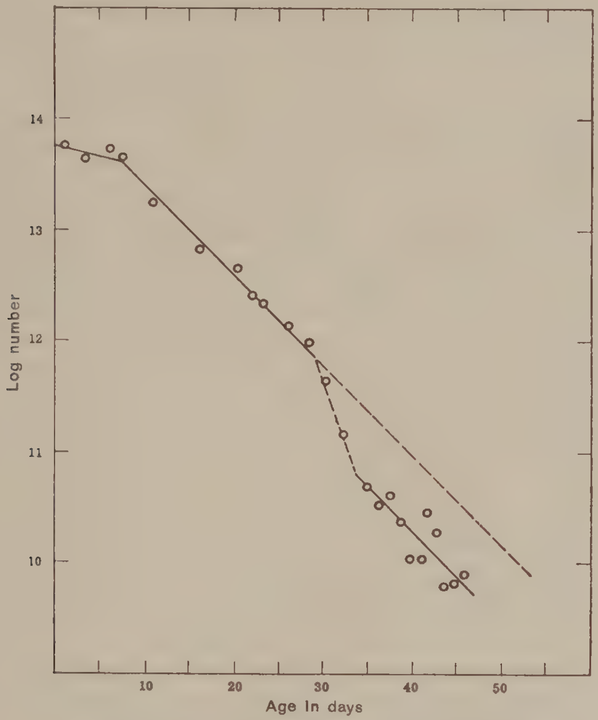

Figure 3. Survival of young stages of Atlantic mackerel.
(From Ahlstrom & Nair, MS, Figure 7.)

Ahlstrom and Nair (MS) have recently re-examined Sette's data. Their analysis differs from his in that they take into account (1) the distribution of eggs and larvae in space and time and (2) the differences between day and night catches, and (3) they have also re-determined larval growth-rate. Their Figure 7 is reproduced here as my Figure 3. Even with these added refinements of analysis, it is apparent that so far as this discussion is concerned, the main features of the estimated survival curve remain the same. Two features are of interest, however. The new estimate supports one of Sette's alternatives, namely, that mortality rates are less for eggs than they are for larvae. This could not be considered a "critical period" in the accepted sense of that term. The apparent short period of low survival rate (which now occurs at about age 31 days owing to the recalculation of growth-rate) is followed by a rate which is similar or identical to that of the earlier period. This lends additional credence to the possibility that the abrupt change in survival rate is not real, but represents increased net-dodging ability after fin formation. The growth data do

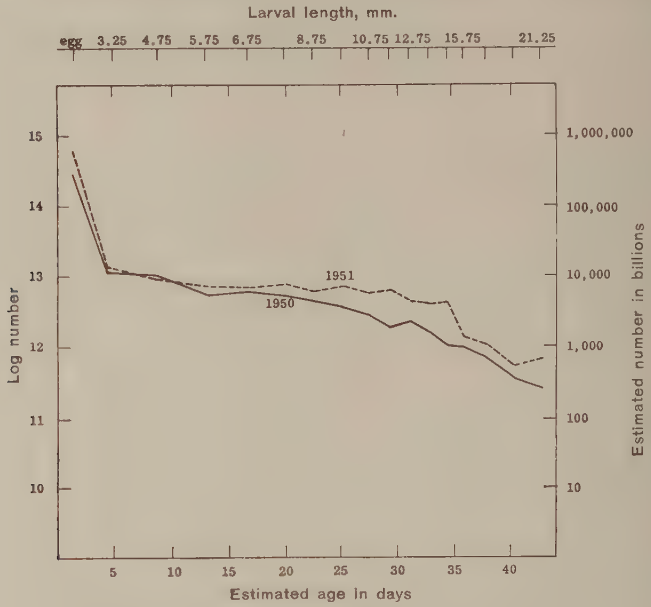

Figure 4. Survival of young stages of Pacific sardine in 1950 and 1951.
(From Ahlstrom, 1954, Figure 31.)

not show a reduced growth-rate in 9—11 mm. larvae (see Figure 5, Ahlstrom and Nair, MS). It is not now possible to make a final choice between the other two possibilities (dodging or mortality); this must be based upon additional observations at sea.

The second study which deals critically with the early survival of a marine fish is that of Sette and Ahlstrom (1948) and Ahlstrom (1954) on the Pacific sardine (*Sardinops caerulea*) of North America. The latter has given survival curves of the 1950- and 1951-classes over the first 45 days of life, during which the larvae attain a length of 21·25 mm. His Figure 31 is reproduced here as my Figure 4. The survival curve of the 1952-class (unpublished data) does not differ in general form from those for the 1950- and 1951-classes.

These curves lead one to infer a high mortality rate between the eggs and the 3·25 mm. larvae, a very low mortality rate between the 3·25 and 14·75 mm. larvae, and a higher mortality rate for larvae larger than 14·75 mm. The yolk-sac of sardine larvae is absorbed by about 4·50 mm. and these curves, therefore, might well be interpreted as exhibiting an early "critical period". However, as Ahlstrom (1954: 56—72) points out, some of the newly hatched larvae are lost through the meshes of the plankton nets. Such loss decreases as the larvae increase in size. While the exact amount of these losses has not yet been determined, present evidence (unpublished) indicates that, for example, only about one-tenth of the 3·25 mm. larvae that enter the net are retained by it. This suggests that the true shape of these survival curves is such that the survival rate increases at a nearly constant rate, as shown by the broken line in Figure 1.

The terminal increase in mortality rate in the sardine survival curves may be real or it may only represent undersampling, such as would be caused by net-dodging by the larger larvae. Only additional observations will indicate which is the correct alternative.

I have been unable to find any other published studies of the early survival of marine fishes which are as comprehensive as those of the Atlantic mackerel and the Pacific sardine. Mr. D. A. Farris, U. S. Fish and Wildlife Service, has in progress a similar study of recent year-classes of the jack mackerel (*Trachurus symmetricus*), but this is not yet sufficiently advanced to estimate the form of the early survival curve.

Mr. A. C. Simpson, Ministry of Agriculture and Fisheries, England, has started a similar study of the plaice (*Pleuronectes platessa*), which has not yet been published. However, he has kindly informed me (personal communication dated 22. January 1954) that in the first year of surveys "... there was no evidence that pointed to a catastrophic mortality at any stage".

Soleim (1940, 1942) has inferred that herring have a "critical period" which coincides with the time of yolk-sac absorption and is the result of a lack of sufficient food. (These papers are in Norwegian; I have been able to read only the English summaries and an English translation of the 1942 paper which is in the library of the International Fisheries Commission, Seattle.) He bases this inference on three sets of observations:— (1) The numbers of larvae taken in plankton hauls (off the coast of Norway) decrease rapidly with increase in length of larvae. (2) Some of the plankton hauls contained, in addition to larvae which had obviously been alive when caught, large numbers of larvae which had obviously been dead some time before capture. These larvae were largely at the stage of yolk-sac absorption. (3) In rearing experiments in the laboratory, large numbers of larvae died following yolk-sac absorption.

The latter need not be considered here, since I am concerned only with the existence of a "critical period" in nature. With reference to (1) above, "numbers as a function of size" survival curves may be misleading as compared to the true "numbers as a function of age" survival curves, since at different sizes of larvae a unit increase in size represents different time periods. But there exists an even more serious objection to Soleim's inference. Unless the area of spawning and area of larval drift are adequately encompassed in time and space, variations arising from sampling deficiencies may appear in the survival curves. Without additional data it will be impossible to ascribe the exact cause of such variations. It is not apparent that the area was adequately encompassed in space and time.

Concerning (2) above, dead larvae taken in plankton hauls may have starved to death, but, alternatively, they may have died as the result of bacterial infection, or any of a number of other causes. Furthermore, the appearance of dead and disintegrating larvae in plankton collections may simply be an artifact. Such larvae are common, for example, in hauls which also contain large numbers of hard, sharp forms, such as pteropods. Damage to the larvae obviously results from contact with the pteropods within the plankton net. A more common source of dead larvae in a given plankton haul is contamination from the previous haul; i.e., inadequate washing of the net between hauls. This appears to be the most likely explanation of the appearance of dead herring in Soleim's hauls. According to his Table 1 (1942: 8), the hauls in which large numbers of dead larvae occurred invariably followed hauls in which large numbers of normal larvae were taken. Dr. E. H. Ahlstrom has informed me (personal communication) that in our studies of the Pacific sardine and other pelagic species, the occurrence of dead larvae in plankton collections always follows inadequate washing of the net between hauls.

Pending additional information, I conclude that this record of dead herring larvae probably represents an artifact. However, the possibility that they died before capture, of starvation or some other cause, is not completely eliminated. For example, dead newly hatched jack mackerel larvae were taken off the coast of southern California at 30°58·5′N. Lat., 120°45′W. Long. on 24. March 1954. In a day-time series of vertically stratified horizontal hauls, jack mackerel larvae were taken at 7 levels from the surface to 67·5 m. The surface haul (0903—0913 PST) contained 73 newly hatched larvae, of which 69 were in a disintegrating condition with missing heads. The haul of 6·8 m. (0935—0946 PST) contained 76 larvae, of which 70 were dead. All other jack mackerel larvae captured at greater depths were of normal appearance. In a similar night-time series, jack mackerel larvae were taken at 5 levels from the surface to 64·2 m. The surface haul (2025—2035 PST) contained 11 newly hatched larvae, of which all were dead. Jack mackerel larvae were absent in the 6·8 m. haul, but 3 in normal condition were taken in the 17·4 m. haul (2120—2130 PST). The cause of this mortality is not known. It was not an artifact arising from inadequate washing of the net between hauls because (1) the net was new and had never been used before the first surface haul and (2) dead larvae were not found in the hauls between the day and night surface hauls. Neither was it the result of thermal stratification at the surface, since during the day series the temperature was 14·1°C. at the surface and 14·0°C. at the 53·2 m. level; during the night series it was 14·3°C. at the surface and 14·1°C. at the 64·2 m. level.

In addition to the Atlantic mackerel, sardine, herring, and jack mackerel data discussed above, there are, scattered through the literature, data (on various species) which are almost, but not quite, suitable for the derivation of survival rates. An example is the information on coalfish (*Gadus virens*) given by Bertelsen (1942). Even though such contributions were not intended to bear on this subject, one cannot help but wish that they were.

## Summary and Conclusions.

Although the "critical period" hypothesis of the cause of fluctuations in year-class sizes of marine fishes was postulated forty years ago, little evidence with which to test the hypothesis has been accumulated since then.

Of the various forms which early survival curves could take, three seem most likely to obtain. These are (1) the "critical period" type, with catastrophic mortalities restricted to a brief period, (2) the form in which the survival rate is increasing at a constant rate, and (3) the form in which survival rate is constant.

Data with which these possibilities may be examined have been published for only one year-class of Atlantic mackerel and two year-classes of Pacific sardine. The Atlantic mackerel data may be interpreted as having relatively constant survival rate, but with a brief period of high mortality (which may be real or may be the result of increased swimming ability of the larvae) at about age 30—40 days. There is no "critical period" at the time of yolk absorption. The Pacific sardine data may be interpreted as having an early "critical period" of high mortality. However, only about one in ten of the smallest larvae were retained by the nets used and it is likely that the true survival rate is increasing at a constant rate.

Records of the natural occurrence of dead larvae may be real or may be artifacts.

The question "Is there a 'critical period' *in nature* in the early life history of marine fishes?" cannot yet be answered categorically. Catastrophic mortalities restricted in time will always remain as a possibility, of course, but the weight of what little evidence there is points toward survival at a constant rate or at a constantly increasing rate, rather than toward the existence of a "critical period".

I have tried to explore the implications of these two general types of mortality (one concentrated in time and the other spread over a longer period) on the hypotheses which biologists will wish to test and, especially, on the methods of testing them. In either case the causes of mortality may be the same. However, in the case where mortalities are severely restricted in time, one might expect that the causes would be more striking or obvious and thus be more readily recognized. Yet, if the mortalities are severely restricted in time, even though obvious, the chances that the observer will make the right observations at the right time and the right place are greatly reduced. If, on the contrary, the mortalities are spread over a longer time, one might expect that the causes would be less striking and not so readily recognized. But the observer will have better chances of making the right observations at the right time and place.

## Acknowledgements.

I wish to thank E. H. Ahlstrom, O. E. Sette, and T. M. Widrig, U. S. Fish and Wildlife Service, and A. C. Simpson, Ministry of Agriculture and Fisheries, England, for critically reading and commenting upon this paper, and George Mattson, U. S. Fish and Wildlife Service, for preparing the figures.

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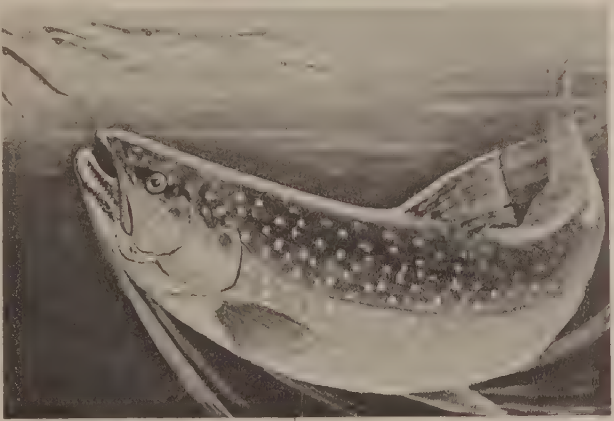
A/S L. IHRICH . COPENHAGUE

# The Lake Trout

## Endangered in the Great Lakes

### JAMES W. MOFFETT

The lake or mackinaw trout (*Salvelinus namaycush*) is native to northern North America from Alaska to the Labrador Peninsula, south to northern New England, headwater lakes of the St. Lawrence and Hudson River systems in New York, the Great Lakes drainage basin, lakes on the northern margin of the Mississippi River watershed in Wisconsin and Minnesota, headwaters of the Columbia and Fraser Rivers and Vancouver Island. It has been introduced into many United States lakes as a game fish.



FROM WILDLIFE CONSERVATION STAMP SERIES

The lake trout is distinguished from its nearest relative, the brook trout, by its strongly forked tail, its gray-spotted body without bright colors, and the lack of light conspicuous edgings on the lower fins. It grows to a large size; 30-pounders are not uncommon. Trout weighing more than 100 pounds have been reported, but fish of this size are presently rare. As a juvenile, the lake trout eats plankton, terrestrial and aquatic insects and occasionally small fishes. As an adult in the Great Lakes, it feeds principally on deep-water ciscoes, muddlers, sticklebacks and smelt. Lake trout prefer deep, cold waters. Some races seldom approach shallow water except at fall spawning time when most move into bouldery shoals to scatter their eggs among the rocks. A few races spawn in streams.

The lake trout is not in danger of extinction except in the Great Lakes. Prior to 1937 this fish reached its maximum development in Lakes Huron, Michigan and Superior. Commercial fishermen in Canada and the United States annually caught an average of 15,375,000 pounds, worth about $7½ million at present prices. A vigorous sports fishery, "deep-sea trolling," was growing rapidly. Beginning in 1939, commercial production began to decline and the sports fishery lost quality, first in Lake Huron, then in Lake Michigan. Now, the decline has started in Lake Superior. By 1953, production in Lake Huron was down to 344,000 pounds and there was no production in United States waters. Lake Michigan produced 22 pounds in 1954. Lake Superior's production was down to about 85 per cent of normal in 1953. Indications are that production in 1954 was near 72 per cent of normal and 1955 catches have been very disappointing.

The major cause of this phenomenal loss of lake trout is the sea lamprey, an eel-like predator which subsists entirely on the blood and body juices of fish. By means of its sucker-like mouth, armed with sharp teeth, the lamprey attaches itself to a victim, rasps a hole into its flesh, and feeds until its appetite is sated or the victim dies. For many years these lampreys, adapted to life in fresh water, have lived in Lake Ontario. But it is only recently that they have become established in the upper lakes by moving around Niagara Falls through the Welland ship canal. Their increase has been meteoric and disastrous. Studies in Lake Michigan show all too clearly that the lake trout is practically extinct. Only 8 were caught in more that 1,000 miles of gill net fished at many locations during the spring of 1955. In 1930-1932, this amount of gear would have taken more than 50,000 fish.

Although the lake trout is still present in considerable numbers in Lake Superior, it appears only a matter of time until history repeats itself. Sea lampreys found it difficult to pass the navigation locks and dam at the outlet of the lake, but by 1946 this barrier was crossed. They are now well established in the lake and their numbers are increasing annually.

Control of the sea lamprey and restoration of the lake trout stocks are inseparable problems. More than ten years of study of practically every phase of the sea lamprey's life and habits and about five years of recent research on the lake trout have been invested in this effort. Some seven years of experi-

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DR. JAMES W. MOFFETT is identified as one of the principal leaders of research projects to control sea lamprey depredation of lake trout within the Great Lakes. He is Chief of U. S. Fish and Wildlife Service's Great Lakes fishery investigations.

mentation with lamprey control schemes have also contributed to the development of some good prospects for restoration.

Electrical weirs across the mouths of streams, into which the sea lamprey must go to spawn, stop migration and break the life cycle. Canada and the United States have installed 72 of these devices on Lake Superior streams. Weirs are on 19 Lake Michigan streams but many more must be built.

A long search for compounds specifically toxic to sea lamprey larvae in streams, and relatively harmless to other fishes, wildlife and man, has yielded several promising leads. Of the more than 5,000 compounds tried, 8 are being tested exhaustively before they are used. If the testing is favorable, applications of this control method can be carried out in 1956. An attack on the lamprey larvae (ammocoetes) is especially desirable because at least five generations are vulnerable at one time before they become parasitic and harmful to the trout.

The where, when, and how of planting lake trout for greatest survival is being learned before restoration is begun. Artificially propagated fish will be expensive, and knowledge properly applied will minimize losses. Such information becomes more valuable in view of the possibility that lake trout eggs may be difficult to obtain in the quantities required once the Lake Superior trout stocks are eliminated. Research has demonstrated the comforting fact that no competitors have usurped the environmental niche of the lake trout. Chances of restoration would face even greater odds if some other species had taken over the waters originally inhabited by them.

On October 11, 1955, with the exchange of instruments of ratification between the United States and Canada, a Great Lakes Fishery Treaty began to function. The commission created by this treaty has sea lamprey control as one of its major obligations. It will also coordinate fishery research so that united efforts can be brought to bear on problems of sustained fishery production.

Even with the development of methods for accomplishing the job, the effort required to restore the lake trout is great. The stakes are high but the economics are sound. Lake trout are needed in the Great Lakes not only to ease the financial stresses of an old and widespread industry, but ultimately to supply millions of man-days of pleasure each year to a rapidly growing population of sports-loving Americans.

*Separates of these and other articles from "Our Endangered Wildlife" are available from the National Wildlife Federation, Washington 12, D. C.*

# NOTES

## Tagged Bigeye Tuna Recovered

A bigeye tuna *(Parathunnus sibi)* tagged by
Pacific Oceanic Fishery Investigations in
North Pacific was recently recaptured by
Japanese during their winter longline oper-
ons in waters north of the Hawaiian Islands,
rking the first known bigeye tag recovery
the Pacific.

The fish was tagged on January 31, 1955, by
U. S. Fish and Wildlife Service research
ssel *John R. Manning* (cruise 23), at 30°
'N, 171°14'W, which is about 360 miles
rtheast of Midway Island, during an albacore
rvey cruise to the North Pacific. The tuna,
ptured by longline, measured 122.3 cm. in
rk length at the time of release.

The recovery was made by a Japanese long-
ne vessel, the *No. 5 Nikko Maru,* of the Nikko
shing Company of Fukushima Prefecture, on
ovember 24, 1955, at 32°41'N, 155°57'W,
pproximately 700 miles due north of Oahu,
H. The straight-line distance between the
oints of release and recovery is 800 miles.
The length of the fish at recapture was 126.8 cm., indicating a net gain in length of 4.5 cm. during the interval of 10 months. In terms of estimated weight this represents a gain of approximately 10 pounds.

This recovery was the first from a total of 82 bigeye tuna tagged and released in North Pacific waters by POFI between January, 1954, and September, 1955. The tag, in excellent condition, together with pertinent information regarding its recovery, was returned to the Pacific Oceanic Fishery Investigations laboratory through the cooperation of Mr. Shoji Ueyanagi of the Nankai Regional Fisheries Research Laboratory of Kochi, Japan. The tag used was a type "G" vinyl plastic tube tag recently developed by the California Department of Fish and Game (Wilson, R. C., 1953, Calif. Fish and Game 39(4):429-442).—*Tamio Otsu and Richard N. Uchida, Pacific Oceanic Fishery Investigations, U. S. Fish and Wildlife Service, Honolulu.*

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# FISHERIES RESEARCH BULLETIN NO. 19

## THE NUTRITION OF TROUT
## CORTLAND HATCHERY REPORT NUMBER 24
## FOR THE YEAR 1955

by
Arthur M. Phillips, Jr.
Floyd E. Lovelace
Henry A. Podoliak
*Branch of Fishery Biology, Fish and Wildlife Service*

Donald R. Brockway
*New York Conservation Department*

George C. Balzer, Jr.
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*With the Assistance of*
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The Cortland Hatchery is operated under cooperative agreement between the New York Conservation Department, The Fish and Wildlife Service of the United States Department of the Interior, and Cornell University.

Published 1956

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## SUMMARY

The absorption of minerals from food and water by brook trout has been studied by means of radioactive tracers. The absorption of cobalt from water is a valid index of metabolic activity. Both high water temperature (51°F.) and low calcium (5 p.p.m.) cause an increase in cobalt absorption. Significant amounts of cobalt are deposited in the gastro-intestinal tract of trout. The gills are the site of absorption.

Starving brook trout absorb less calcium from water than feeding trout. After 1 week of starvation the metabolic activity of the fish (as measured by calcium absorption) is greater than after 3 weeks. The distribution in the body of calcium absorbed from water is influenced by the dietary level and vice versa. Brook trout absorb more calcium from low level than from high level calcium diets. The osmoregulatory function of calcium is confirmed.

A chemical comparison of hatchery and wild brook trout indicates that wild fish are superior. More true flesh production (ash and protein) is found in the bodies of wild trout. A comparison of protein conversions speculates that natural food is more efficient than hatchery food. The protein conversion for hatchery food is twice that of natural food.

A chemical embryological study of developing brown trout eggs has shown changes in protein, fat, and water content during incubation. The ash and iodine number of the fat remain unchanged. The wet weight of the egg decreases during incubation. Explanations are offered for the results.

The need of brown trout for the vitamins inositol, $B_{12}$, pyridoxine, niacin choline, riboflavin, folic acid, and biotin are established.

# INTRODUCTION

The research program of the Cortland Experimental Hatchery for the year 1955, consists of four independent studies. Three of these studies are a continuation of previous work and the fourth is a new undertaking. The studies are as follows:

1. The Absorption of Minerals from Food and Water by Brook Trout. This study is a continuation of previous work that has established the direct absorption of minerals from water by brook trout. The present experiments are designed to measure some of the chemical and physical factors that effect the absorption of cobalt from water and the absorption of calcium from water and food.
2. A Chemical Comparison of Hatchery and Wild Brook Trout. This study is an extension of the completed work on the chemical comparison of hatchery and natural trout food. The present series of experiments chemically compare the bodies of hatchery and wild trout, and speculatively evaluate the efficiency of wild and hatchery foods in terms of the chemistry of the trout body.
3. The Chemistry of Developing Brown Trout Eggs. This is a new study designed to determine the chemistry of developing trout eggs so that a basis may be obtained for future studies involving the chemical and physical factors that effect the viability and production of trout eggs in hatcheries.
4. The Vitamin Requirement of Brown Trout. Past studies have established the requirements of brook and rainbow trout for members of the vitamin B complex. The present studies produce similar information that will be useful in formulating hatchery diets for brown trout.

# THE ABSORPTION OF MINERALS FROM WATER AND FOOD BY BROOK TROUT

At the Cortland Experimental Hatchery, a continued integrated program of general quantitative and qualitative studies on wild and hatchery fish, natural and hatchery food, and the chemical content of the water is supplemented with specialized radioactive tracer studies which attempt to correlate the minerals of food and water and their interactions, with the metabolism of trout under the widely diverse conditions that trout must survive.

The production capacities of hatchery and natural waters of trout are determined by the best available judgments of their physical and chemical characteristics and by the type and quantity of food presented or naturally present in the waters.

The relationship of dissolved minerals to the food chain is confounded by the direct absorption of these minerals by the higher forms of animal life, thus bypassing the lower forms of life in the chain. The materials for all metabolic processes (growth, maintenance, and body functions) must be provided by the food and water. In addition aquatic animals must maintain an osmotic equilibrium with the surrounding water, which is a function of metabolism although not commonly so recognized. Therefore, trout are in competition with others forms of life in natural waters for these minerals and in hatcheries they are dependent upon the fish-culturist's skill in supplying or supplementing them.

Fresh water fish have their body surfaces continually in contact with a medium more dilute in concentration of dissolved minerals than the fluids of their bodies. Active absorption of ions from the water is necessary to maintain an osmotic equilibrium in the body and to prevent a depletion of ions from the body. The site of most active ingress of minerals is the gill, where the ions are taken from the flow of water used in respiration, and are rapidly transferred throughout the trout's body. Over a period of two or three days, a significant deposition of absorbed ions can be measured. Since the food of trout contains a generous supply of mineral nutrients, the use of absorbed minerals for structural purposes may be of a supplemetal but none the less important nature. Through normal metabolism there is a continual loss of ions through the gills and the urinary and intestinal tracts.

This absorption is not a simple quantitative chemical interchange at the gill surface. The rate of absorption is governed by the metabolic activity of the fish and is an active process with a corresponding expenditure of energy. According to Krogh (1939), calcium ions have a specific osmoregulatory function on many fresh water organisms. Previous experiments at this laboratory (Phillips et al, 1954), show an increased rate of absorption of calcium ions from very dilute solutions, indicating an osmoregulatory function for calcium in trout.

To study further the role of dissolved and ingested minerals in trout physiology, this group of experiments has been designed to measure:

1. The absorption of cobalt from water and the validity of the absorption of $cobalt^{60}$ as a measure of metabolic activity.
2. The validity of ascribing to calcium ions in water an osmoregulatory action for trout.
3. To confirm the metabolic effect of low calcium water by comparison with known metabolic effects of cold and warm waters.
4. To determine the significance of any difference in the amounts and distribution of dissolved minerals absorbed by starving and feeding trout.
5. To correlate high and low calcium content of both feed and waters.

## Factors Affecting the Quantitative Absorption of Cobalt from Water

The importance of trace elements in the nutrition of higher animals is well established. For the most part, analytical values for the trace elements in waters, foods of trout, and the bodies of trout are lacking. The fact that radiocobalt is absorbed from water (Phillips et al, 1954) is sufficient to justify further study.

Radioactive $cobalt^{60}$ offers an attractive tool for research on the trace elements of trout. As little as $10^{-12}$ gram of radiocobalt in the trout body is readily detectable with the Geiger-Müller tube. By varying the calcium concentration and the temperature of the water labeled with radiocobalt, changes in metabolic activity can be measured by the amount of $cobalt^{60}$ absorbed. The confounding effect of measuring in the trout body that element (calcium) which is being used to stimulate the differences sought is thus eliminated. Further, a possible osmoregulatory action of any ion, rather than calcium specifically, can be tested using cobalt as the test mineral.

## Experimental

Since a radioisotope of an element has the same chemical properties as the normal element, the thorough mixing of the isotope with the normal element present in food and water labels that particular element and allows its path through the body of a fish to be traced quantitively by simple measurement of radioactivity. The amount of radioactivity can be kept low, causing no derangement of normal life processes.

The factorial design of Fisher (1937) was used for these experiments. As in the past (Phillips et al, 1954) the standard for comparison was the calcium and cobalt concentrations found in the Cortland hatchery spring water.

The experimental conditions were the following:

1. All experimental waters contained the specified concentration of cobalt and calcium in either distilled or spring water.
2. Two concentrations of cobalt labeled with cobalt$^{60}$ were used: 0.25 micrograms per liter (concentration of Cortland spring water) and 2.5 micrograms per liter.
3. Two concentrations of calcium were used: 50 p.p.m. (concentration of Cortland spring water) and 5 p.p.m.
4. Two temperatures were used: 51°F., (average temperature of the water in the production unit of the Cortland Hatchery) and 36°F.
5. Four geometrically increasing times of exposure to the labeled waters were used: 4, 8, 16 and 32 hours. These span the most significant time intervals of previous absorption studies at this laboratory.
6. The fish were randomly chosen brook trout ranging from 8 to 16 grams in weight.
7. The distribution of both total and absorbed cobalt of the gill, gastro-intestinal tract, and the remainder of the fish was measured.

To provide a relatively stable metabolic activity by acclimating the trout to aquaria conditions, changes in water composition, and differences in water temperature, and to insure elimination of waste products from feeding prior to the experiment, all trout were starved for 64 hours in conditioning aquaria containing water physically and chemically identical with the experimental water but lacking radioactivity. Pure radiocobalt chloride was used to establish the labeled concentrations in the experimental water. Standardized solutions of calcium chloride were used for the conditioning and experimental waters to vary the calcium content.

## Procedure

A population (1,000 trout) of fingerling brook trout was taken from a hatchery raceway and placed in a holding trough in the hatchery building. The fish were fed the standard hatchery diet.

For conditioning the trout in each experimental run, 10 fish were netted from the holding trough and placed in 3,000 cc of aerated, continuously filtered, non-radioactive water, in a 4,000 cc glass beaker, held in the constant temperature room of this laboratory. The room temperature was 51°F. Immersion of the beaker in a large pan of chopped ice obtained a temperature of 36°F. when required. The fish were not fed during the conditioning period. After a lapse of 64 hours the 10 fish were rapidly transferred to 3,000 cc of aerated, labeled experimental water in a 4,000 cc glass beaker.

At the requisite time interval, the fish were killed and separated into two groups of five fish each. Each group of trout was dissected, and a pooled sample of the parts of the five trout was placed into individual tared containers (25 ml. Erlenmeyer flasks for the gills; 50 ml. Erlenmeyer flasks for the gastro-intestinal tracts; No. 2/0A porcelain dishes for the remainders of the trout). After weighing the samples, one milligram of cobalt sulphate was added to each container as an inert carrier. The samples were dried at 100°C., ignited in a muffle furnace at 600°C., and finally wet ashed with 1 to 3 ml. of concentrated nitric acid. The resultant ash was taken up in 5 to 10 ml. of 1:1 nitric acid, and this ash solution was stored in plastic tubes. Aliquots of the ash solution were measured for radioactive cobalt following the method previously described (Phillips et al, 1954).

Since five fish were too small a sample for quantitative analysis of total cobalt, 30 fish were netted from the holding trough at intervals during the experiment and dissected in the same manner as the fish which had been exposed to labeled cobalt. The samples for analysis were dried overnight at 100°C. The ashing and preliminary separation of cobalt followed the procedure of Saltzman (1955), using 2-nitroso-1-naphthol to form the cobalt complex. Cobalt was determined colorimetrically by adapting this procedure to the color formed by 2-nitroso-1-naphthol and reading in a Lumetron colorimeter.

## Discussion

Differences were established by analysis of variance. Tables 1a, 1b, 1c, and 1d present the summarized data of this experiment. Of the major variables, significant differences result from changes in cobalt concentration, calcium concentration, and the temperature of the water. No significant difference occurs when spring water replaces distilled water. A significant difference in distribution of absorbed cobalt to the parts and a significant increase in the amount of absorbed cobalt distributed are found with an increase in time of exposure to radioactive cobalt. The distribution within each time interval is independent of the water temperature and the calcium concentration of the water, but is significantly influenced by the amount of cobalt available to the fish from the water.

**Effect of cobalt level:** More cobalt is absorbed from the water by the fish when more is made available for absorption. The concentration of 0.25 micrograms per liter represents the physiological dose. This concentration is of the same order of magnitude as the normal level of cobalt in water in which the fish has been living. A tenfold increase (2.5 micrograms per liter) is a relatively massive dose, but does not approach toxicity. After 4 hours of exposure to labeled cobalt in the water, the ratio of the amounts of absorbed cobalt in the trout is of the same order of magnitude as the respective concentrations of cobalt in the waters in which the trout are held. The ratio decreases as the time of exposure to labeled cobalt in the water increases.

Two interpretations may be made of the physiological significance of this decrease. One, the massive dose provides an excess of labeled cobalt in water beyond the requirement of brook trout, and the trout possess a selective mechanism for limiting the amount absorbed from the water to the amount required. Two, the physiological dose in the water does not supply the cobalt requirement of the starving trout, and an active increased uptake (per unit of time) occurs. It is evident that the absorption of labeled cobalt from the water is not a simple chemical interchange, but is regulated by the trout. The significant interactions (Chart I) of the cobalt concentrations of the water with the calcium concentration of the water, the water temperature, the time of exposure to the labeled water, and the particular part of the fish involved, indicate a complex chain of environmental adjustments.

Although it is intriguing to find one of the trace elements which, in animal nutrition, has a regulatory function similar to that of vitamins (Comar, 1948), being used by brook trout, the role of cobalt remains unknown. Even though the specific physiological function of cobalt in trout is unknown, the influence of other factors on the cobalt distribution within the trout provides evidence of physiological processes in which cobalt may be concerned.

TABLE 1a. Absorbed cobalt (millimicrograms), wet weight of gill tissues (grams), and ratio of absorbed cobalt to wet weight of gill tissues of brook trout after exposure to labeled cobalt in aquarium water.

| Concentration of | | | Temperature of aquarium water | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 51° F. | | | | 36° F. | | | |
| | | | Time in hours | | | | Time in hours | | | |
| Cobalt micrograms[1] | Calcium P.P.M. | | 4 | 8 | 16 | 32 | 4 | 8 | 16 | 32 |
| | | | Distilled water | | | | | | | |
| 0.25 | 5.0 | Absorbed cobalt | 0.0674 | 0.0825 | 0.1594 | 0.2646 | 0.0330 | 0.0586 | 0.0433 | 0.1599 |
| | | Wet weight gills | 0.174 | 0.202 | 0.218 | 0.274 | 0.218 | 0.265 | 0.212 | 0.272 |
| | | Ratio | 0.387 | 0.408 | 0.731 | 0.966 | 0.151 | 0.221 | 0.205 | 0.588 |
| 0.25 | 50.0 | Absorbed cobalt | 0.0419 | 0.0377 | 0.0543 | 0.0806 | 0.0191 | 0.0298 | 0.0338 | 0.0503 |
| | | Wet weight gills | 0.246 | 0.272 | 0.285 | 0.235 | 0.237 | 0.269 | 0.251 | 0.252 |
| | | Ratio | 0.170 | 0.139 | 0.191 | 0.343 | 0.081 | 0.111 | 0.135 | 0.200 |
| 2.50 | 5.0 | Absorbed cobalt | 0.7105 | 1.2237 | 1.0676 | 1.5154 | 0.3022 | 0.4029 | 0.4092 | 0.6957 |
| | | Wet weight gills | 0.194 | 0.219 | 0.200 | 0.234 | 0.229 | 0.223 | 0.253 | 0.191 |
| | | Ratio | 3.657 | 5.585 | 5.343 | 6.487 | 1.322 | 1.805 | 1.620 | 3.652 |
| 2.50 | 50.0 | Absorbed cobalt | 0.1404 | 0.3659 | 0.4941 | 0.7446 | 0.1791 | 0.1799 | 0.3057 | 0.6069 |
| | | Wet weight gills | 0.263 | 0.207 | 0.241 | 0.241 | 0.266 | 0.238 | 0.265 | 0.223 |
| | | Ratio | 0.534 | 1.764 | 2.054 | 3.085 | 0.673 | 0.751 | 1.155 | 2.720 |
| | | | Spring water | | | | | | | |
| 2.50 | 50.0 | Absorbed cobalt | 0.3001 | 0.5764 | 0.5207 | 0.9457 | 0.2021 | 0.3514 | 0.2951 | 0.3800 |
| | | Wet weight gills | 0.240 | 0.291 | 0.248 | 0.276 | 0.301 | 0.290 | 0.292 | 0.305 |
| | | Ratio | 1.251 | 1.981 | 2.104 | 3.425 | 0.672 | 1.214 | 1.009 | 1.243 |

[1] Micrograms per liter

TABLE 1b. Absorbed cobalt (millimicrograms), wet weight of digestive tract (grams), and ratio of absorbed cobalt to wet weight of digestive tract of brook trout after exposure to labeled cobalt in aquarium water.

| Concentration of | | | Temperature of aquarium water | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 51° F. | | | | 36° F. | | | |
| | | | Time in hours | | | | Time in hours | | | |
| Cobalt micro-grams[1] | Calcium P.P.M. | | 4 | 8 | 16 | 32 | 4 | 8 | 16 | 32 |
| | | | Distilled water | | | | | | | |
| 0.25 | 5.0 | Absorbed cobalt | 0.0034 | 0.0068 | 0.0209 | 0.0697 | 0.0013 | 0.0035 | 0.0077 | 0.0188 |
| | | Wet weight tract | 0.430 | 0.505 | 0.485 | 0.547 | 0.602 | 0.712 | 0.643 | 0.759 |
| | | Ratio | 0.008 | 0.013 | 0.043 | 0.127 | 0.002 | 0.005 | 0.012 | 0.025 |
| 0.25 | 50.0 | Absorbed cobalt | 0.0029 | 0.0031 | 0.0100 | 0.0338 | 0.0009 | 0.0020 | 0.0057 | 0.0177 |
| | | Wet weight tract | 0.438 | 0.558 | 0.495 | 0.478 | 0.586 | 0.696 | 0.678 | 0.661 |
| | | Ratio | 0.007 | 0.006 | 0.020 | 0.071 | 0.001 | 0.003 | 0.008 | 0.027 |
| 2.50 | 5.0 | Absorbed cobalt | 0.0498 | 0.0908 | 0.1433 | 0.3878 | 0.0126 | 0.0234 | 0.0695 | 0.0767 |
| | | Wet weight tract | 0.474 | 0.462 | 0.420 | 0.486 | 0.653 | 0.692 | 0.728 | 0.642 |
| | | Ratio | 0.105 | 0.197 | 0.341 | 0.797 | 0.019 | 0.034 | 0.095 | 0.120 |
| 2.50 | 50.0 | Absorbed cobalt | 0.0091 | 0.0246 | 0.1480 | 0.1239 | 0.0108 | 0.0122 | 0.0296 | 0.0556 |
| | | Wet weight tract | 0.512 | 0.493 | 0.394 | 0.492 | 0.716 | 0.691 | 0.684 | 0.670 |
| | | Ratio | 0.018 | 0.050 | 0.375 | 0.252 | 0.015 | 0.017 | 0.043 | 0.083 |
| | | | Spring water | | | | | | | |
| 2.50 | 50.0 | Absorbed cobalt | 0.0255 | 0.0615 | 0.0700 | 0.1339 | 0.0081 | 0.0144 | 0.0227 | 0.0481 |
| | | Wet weight tract | 0.556 | 0.669 | 0.527 | 0.581 | 0.781 | 0.760 | 0.793 | 0.840 |
| | | Ratio | 0.046 | 0.092 | 0.133 | 0.234 | 0.010 | 0.019 | 0.029 | 0.057 |

[1] Micrograms per liter

TABLE 1c. Absorbed cobalt (millimicrograms), wet weight of remainder (grams), and ratio of absorbed cobalt to wet weight of remainder of brook trout after exposure to labeled cobalt in aquarium water.

| Concentration of | | | Temperature of aquarium water | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 51° F. | | | | 36° F. | | | |
| | | | Time in hours | | | | Time in hours | | | |
| Cobalt micro-grams[1] | Calcium P.P.M. | | 4 | 8 | 16 | 32 | 4 | 8 | 16 | 32 |
| | | | Distilled water | | | | | | | |
| 0.25 | 5.0 | Absorbed cobalt | 0.1073 | 0.2342 | 0.3383 | 0.8740 | 0.0678 | 0.1203 | 0.2236 | 0.3252 |
| | | Wet weight remainder | 4.001 | 4.648 | 5.045 | 5.320 | 5.702 | 7.028 | 6.195 | 7.386 |
| | | Ratio | 0.027 | 0.050 | 0.067 | 0.164 | 0.012 | 0.017 | 0.036 | 0.044 |
| 0.25 | 50.0 | Absorbed cobalt | 0.1970 | 0.0983 | 0.1444 | 0.2379 | 0.0726 | 0.0934 | 0.1259 | 0.1500 |
| | | Wet weight remainder | 4.456 | 5.096 | 4.998 | 4.852 | 5.673 | 7.200 | 6.859 | 6.869 |
| | | Ratio | 0.044 | 0.019 | 0.029 | 0.049 | 0.013 | 0.013 | 0.018 | 0.022 |
| 2.50 | 5.0 | Absorbed cobalt | 1.1888 | 2.0166 | 2.3836 | 4.9225 | 0.7699 | 1.2177 | 1.2126 | 2.0930 |
| | | Wet weight remainder | 4.255 | 4.999 | 4.472 | 5.013 | 6.194 | 6.340 | 7.055 | 5.651 |
| | | Ratio | 0.279 | 0.403 | 0.533 | 0.982 | 0.124 | 0.192 | 0.172 | 0.370 |
| 2.50 | 50.0 | Absorbed cobalt | 1.2810 | 0.7604 | 0.8744 | 1.4966 | 0.6076 | 0.5556 | 0.6234 | 1.0884 |
| | | Wet weight remainder | 4.989 | 4.924 | 4.814 | 5.051 | 6.759 | 6.278 | 6.805 | 6.214 |
| | | Ratio | 0.256 | 0.154 | 0.182 | 0.296 | 0.090 | 0.088 | 0.092 | 0.176 |
| | | | Spring water | | | | | | | |
| 2.50 | 50.0 | Absorbed cobalt | 0.7897 | 0.8567 | 0.7565 | 1.0556 | 0.9075 | 0.4539 | 1.0186 | 1.1606 |
| | | Wet weight remainder | 5.248 | 6.400 | 5.531 | 6.002 | 7.559 | 7.647 | 8.062 | 8.361 |
| | | Ratio | 0.150 | 0.134 | 0.137 | 0.176 | 0.120 | 0.059 | 0.126 | 0.139 |

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[1] Micrograms per liter

TABLE 1d. Absorbed cobalt (millimicrograms), wet weight of whole fish (grams), and ratio of absorbed cobalt to wet weight of whole brook trout after exposure to labeled cobalt in aquarium water.

| Concentration of | | | Temperature of aquarium water | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 51° F. | | | | 36° F. | | | |
| Cobalt micrograms[1] | Calcium P.P.M. | | Time in hours | | | | Time in hours | | | |
| | | | 4 | 8 | 16 | 32 | 4 | 8 | 16 | 32 |
| | | | Distilled water | | | | | | | |
| 0.25 | 5.0 | Absorbed cobalt | 0.1781 | 0.3235 | 0.5186 | 1.2083 | 0.1021 | 0.1824 | 0.2746 | 0.5039 |
| | | Wet weight whole fish | 4.605 | 5.354 | 5.748 | 6.141 | 6.522 | 8.005 | 7.049 | 8.417 |
| | | Ratio | 0.039 | 0.060 | 0.090 | 0.197 | 0.016 | 0.023 | 0.039 | 0.060 |
| 0.25 | 50.0 | Absorbed cobalt | 0.2418 | 0.1391 | 0.2087 | 0.3523 | 0.0926 | 0.1252 | 0.1654 | 0.2180 |
| | | Wet weight whole fish | 5.140 | 5.926 | 5.778 | 5.565 | 6.496 | 8.166 | 7.787 | 7.781 |
| | | Ratio | 0.047 | 0.024 | 0.036 | 0.063 | 0.014 | 0.015 | 0.021 | 0.028 |
| 2.50 | 5.0 | Absorbed cobalt | 1.9491 | 3.3311 | 3.5945 | 6.8257 | 1.0847 | 1.6440 | 1.6913 | 2.8627 |
| | | Wet weight whole fish | 4.923 | 5.680 | 5.092 | 5.733 | 7.076 | 7.255 | 8.036 | 6.484 |
| | | Ratio | 0.396 | 0.587 | 0.706 | 1.191 | 0.153 | 0.227 | 0.210 | 0.442 |
| 2.50 | 50.0 | Absorbed cobalt | 1.4305 | 1.1509 | 1.5165 | 2.6651 | 0.7975 | 0.7467 | 0.9587 | 1.7509 |
| | | Wet weight whole fish | 5.764 | 5.624 | 5.449 | 5.785 | 7.741 | 7.207 | 7.753 | 7.108 |
| | | Ratio | 0.248 | 0.205 | 0.278 | 0.461 | 0.103 | 0.104 | 0.124 | 0.246 |
| | | | Spring water | | | | | | | |
| 2.50 | 50.0 | Absorbed cobalt | 1.1153 | 1.4946 | 1.3432 | 2.1352 | 1.1177 | 0.8197 | 1.3364 | 1.5887 |
| | | Wet weight whole fish | 6.043 | 7.360 | 6.305 | 6.859 | 8.640 | 8.697 | 9.147 | 9.508 |
| | | Ratio | 0.185 | 0.203 | 0.214 | 0.311 | 0.129 | 0.094 | 0.146 | 0.167 |

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[1] Micrograms per liter

**Effect of temperature:** Following van't Hoff's law, an increase in the temperature of the water should cause an increased metabolic activity in fish living in the water. Since the absorbed cobalt from the water is actively metabolized by the trout, raising the water temperature 15°F. (from 36 to 51°F.) should cause an increase in the amount of absorbed cobalt distributed to the parts of the trout. This difference does appear after 4 hours of exposure to the temperature difference and, after 32 hours of exposure, is of the order of a three fold increase in the amount of cobalt absorbed from the labeled water.

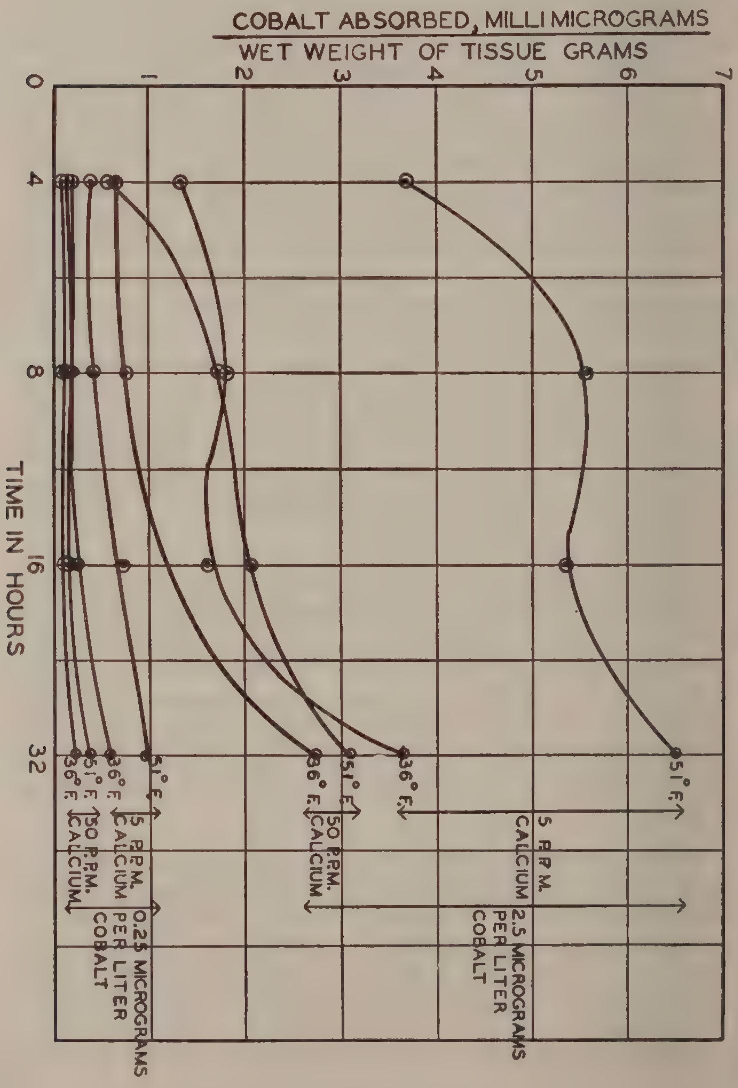
A comparison is possible with the metabolic studies upon trout (Phillips et al, 1954) in which the fish are held in an aquaria and the accumulation of excretory ammonia in the water and the oxygen remaining in the water are measured after various intervals for a temperature change from 36°F. to 48°F. In the experiments of Phillips et al (1954) brook trout of comparable size and starved for a comparable period (67 hours), after 4 hours in the aquarium 1.3 times as much ammonia accumulates in the warmer water and 1.8 times as much oxygen remains in the cooler water. After 8 hours the ammonia is twice as high in the warmer water and the oxygen 1.7 times as high in the cooler water. In terms of metabolic activity the results of the previous and present experiments are similar. Generally the use of labeled cobalt in water to trace the level of metabolic activity appears valid, though the previous work is not strictly comparable because of the significance of numbers of fish, size of the aquaria, and other physical factors.

**Effect of calcium level:** Most significantly, the amount of absorbed cobalt from the water appearing in the whole fish after 32 hours of exposure is slightly greater at the lower water temperature (36°F.) with a concentration of 5 p.p.m. calcium, than at the higher water temperature (51°F.) with a calcium concentration of 50 p.p.m. The effect of decreasing calcium concentration is independent of the time intervals. At all time intervals more cobalt is absorbed in the low calcium water. This increase is approximately three fold in magnitude.

**Effect of minerals:** When normal Cortland spring water replaces distilled water, no significant influence is found from the added minerals (minerals normally present in the spring water) on the absorption and distribution of labeled cobalt from the water. There is no significant interaction of these added minerals with the temperature of the water or the time of exposure.

**Effect of parts:** Table 2 is a statistical summary of the total

CHART I. Cobalt absorbed (millimicrograms) per gram of gill tissue by brook trout immersed in aquaria of different concentrations of cobalt and calcium, at two water temperatures (51°F. and 36°F.).



and absorbed cobalt in the parts of brook trout. The percentage of the total absorbed cobalt contained in a specific part is designated as the distribution of absorbed cobalt. The percentage of total cobalt in a specific part is designated as the distribution of total cobalt. The ratio of these two distributions is designated as the distribution ratio and will equal one if the amount of cobalt contained in a specific part determines the amount of absorbed cobalt to be distributed to that part. If the distribution ratio for a specific part is larger than one, that part contains proportionately more cobalt.

The large distribution ratio for the gill sample indicates the gills as the site of absorption. Sites of transportation, deposition, or elimination of absorbed cobalt are indicated by the lower distribution ratio of the gastro-intestinal tract and the remainder of the fish. Most of the absorbed cobalt and most of the total cobalt appears in the remainder of the trout. Although the length of exposure to labeled cobalt in the aquaria is short, fully 0.5 percent of the trout's cobalt is labeled cobalt from the aquaria.

**Effect of time:** Since the gills are the site of active ingress of ions from the water, the graphical presentation of the cobalt absorbed per gram of gill tissue (Chart II) illustrates the effect of increasing times of exposure on the major variables. Similar, but less dramatic, curves are obtained for the other two parts. At 32 hours, the level of absorbed cobalt in the gills progressively reflects the concentration of cobalt in the water, and the metabolic effects of water temperature

**TABLE 2. Statistical summary of the distribution of total and absorbed cobalt in parts of brook trout.**

| Measurement | Gills | Digestive tract | Remainder | Whole fish |
|---|---|---|---|---|
| Cobalt absorbed, millimicrograms | 0.330 | 0.046 | 0.828 | 1.204 |
| Total cobalt, micrograms | 0.028 | 0.035 | 0.175 | 0.238 |
| Ratio of: $\frac{\text{Cobalt absorbed, millimicrograms}}{\text{Total cobalt, micrograms}}$ | 11.8 | 1.3 | 4.7 | 5.1 |
| Distribution of absorbed cobalt (percent of absorbed cobalt in part). | 27.41 | 3.82 | 68.77 | — |
| Distribution of total cobalt (percent of total cobalt in part). | 11.76 | 14.65 | 73.59 | — |
| Distribution ratio: $\frac{\text{Percent of absorbed cobalt}}{\text{Percent of total cobalt}}$ | 2.33 | 0.26 | 0.93 | — |

and calcium concentration on the absorbed cobalt levels, in the two concentrations of cobalt. The interaction between temperature of the water and calcium concentration in the water is shown by the definite minima in the curves for the low concentration of calcium in the water at either temperature. These minima are not as evident for the physiological dose of cobalt in the water (0.25 micrograms per liter), giving weight to the belief that the massive dose (2.5 micrograms per liter) exceeds the cobalt requirement of brook trout, and requires a physiological adjustment within the trout. The effect of this adjustment is magnified under conditions of increased metabolism.

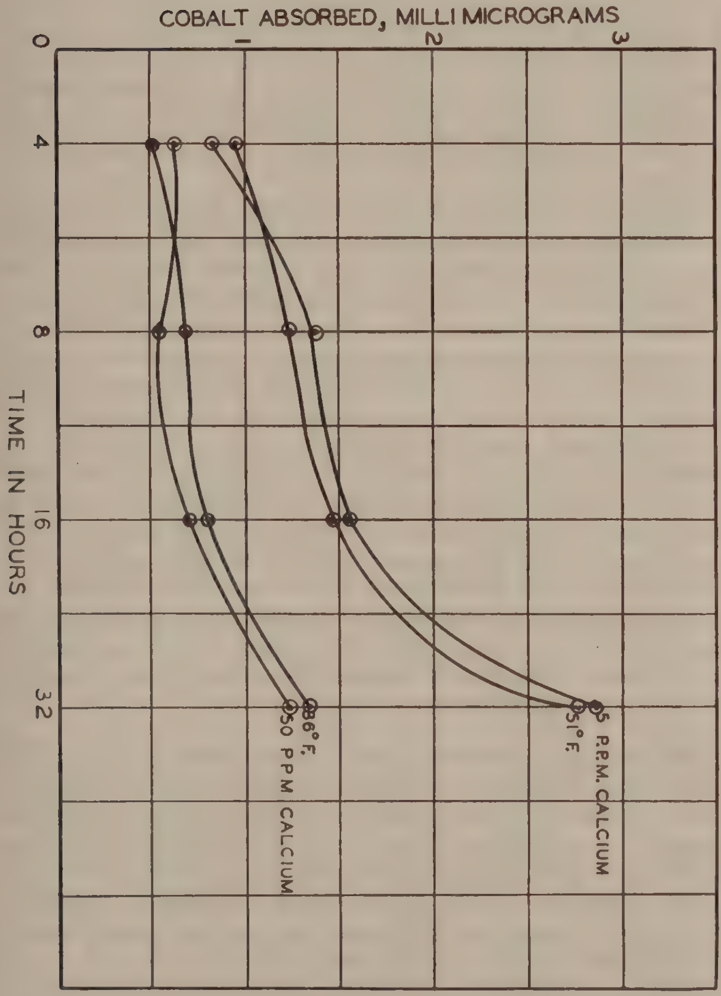
**Osmoregulatory effect of the calcium ion:** Establishing the absorption of cobalt from Cortland spring water as the normal, no change in this absorption is noted when all the minerals normally present, except calcium, are absent. The controlled uptake of cobalt ions by the permeable gill surface, which is the result of ionic regulation, is maintained by having the calcium ion present in distilled water at the concentration usually found in the Cortland spring water (50 p.p.m.). Krogh (1939) postulates that fresh water fish possess special mechanisms for ion absorption, and that the osmoregulatory effect of calcium ions on many other aquatic animals may also exist for fish.

Later studies have shown the specific influence of the calcium ion on the fertilization of aquatic eggs (Yamamoto, 1954; Okada, 1950; Yanagimachi and Kanoh, 1953). These workers note that a calcium free media prevents activation of the spermatazoa and results in unfertilized eggs. Other studies on the effect of calcium on aquatic organisms have been reported. Holm-Jensen (1948) states that to obtain ionic equilibrium for a culture of daphnia, a minimum of 4.5 to 9.0 p.p.m. of calcium must be present in the water; Mast and Pace (1939) report that normal growth and cell division in paramecia requires calcium ions in the water; and Kriszat (1951) finds that the cortical layer of amoeba is softened in calcium free water.

Since 50 p.p.m. calcium in distilled water prevents a breakdown of the ionic regulation of brook trout, it appears that the calcium content of waters, because of an osmoregulatory effect, is of great significance.

**Metabolic effects:** Metabolic activity increases as the concentration of the calcium ion decreases from 50 to 5 p.p.m. A graphical presentation (Chart II) shows that this increase parallels the effect of a 15°F. temperature rise of the water on the total absorption of

CHART II. Summarized effect of a change in calcium concentration of the water and of a change in water temperature on the absorption of labeled cobalt by brook trout.



labeled cobalt over a 32-hour period. Further, the magnitude of a change from 50 to 5 p.p.m. calcium is greater, after 4 hours, than the magnitude of a change from 36°F. to 51°F. A metabolic change should establish a constant rate of metabolism with time. Once a steady state is reached and there are no outside interferences, the increased metabolic activity is a function of the fish mechanism, and a constant rate of metabolism will occur—hence, the importance of the complete lack of a primary interaction of the water temperature and the calcium concentration of the water with the time of exposure. With either of these two variables, once a steady metabolic rate is established, it remains so. No such constancy is noted for the interaction of the cobalt concentration of the water with the time of exposure.

Prosser et al (1945) note an increased uptake of strontium$^{89}$ and of fission mixture by goldfish from pond water low in calcium (1.8 p.p.m.) compared to pond water higher in calcium (36 p.p.m.). This is described as an ion-similarity effect between the strontium and calcium. However, in view of the present experiments and the work of Krogh (1939), this should be considered the result of increased metabolic activity of the fish in pond water of low calcium concentration.

The increased absorption of labeled cobalt from water low in calcium concentration is caused by an increase in metabolism, where the trout expends energy to actively oppose the osmotic inflow of water from the dilute solution, and is capable of maintaining a steady state when the dilute, hypotonic solution contains calcium. Most significant for trout culture is that this increase in metabolism is not noted when all of the other minerals (except labeled cobalt ions) are reduced to zero concentration (distilled water). Fifty p.p.m. calcium in the water is sufficient to maintain normal metabolism as well as normal osmotic regulation.

**Distribution of absorbed cobalt:** The distribution of absorbed cobalt under the various experimental conditions is shown in Table 3. In warm water a larger percentage appears in the gills under the increased metabolism caused by the lower concentration of calcium in the water. The distribution ratio (Table 4) for the gills remains constant with time as does the distribution ratio for the remainder. A steady increase in the distribution ratio for the gastro-intestinal tract discloses the sites of deposition within the trout body.

Referring to Tables 1a, 1b, 1c, and 1d, it is seen that the most outstanding influence of increasing the temperature and lowering the calcium content of the water is one of the absorbed cobalt appearing in the gastro-intestinal tract, relatively more being distributed to these tissues under conditions of higher water temperature and low calcium concentration. If the metabolized cobalt is deposited in the gastro-intestinal, an increased metabolic activity necessarily requires an increased amount of cobalt to appear in these tissues. If the function of the gill is the absorption of the cobalt ion from water, and if the function of the remainder is the transportation of the cobalt ion within the body fluids, an increase in the rate of absorption and the rate of distribution would not reflect as great an increase of absorbed cobalt appearing in these two tissues at any one time. More labeled cobalt passing through the body at a faster rate would not cause as great an increase in the gill and remainder tissues as a faster rate of deposition will cause for the gastro-intestinal tract. An increase of the rate of absorbed cobalt distribution would cause the difference shown in Table 3. Definite storage of cobalt in the gastro-intestinal tract is indicated.

Chart III is a statistical graph which plots the effects on the dis-

**TABLE 3.** Distribution of absorbed cobalt in percent to parts of brook trout.

| Part | | Distilled water | | | | Spring water |
|---|---|---|---|---|---|---|
| | Cobalt micro-grams per liter | 0.25 | | 2.5 | | 2.5 |
| | Calcium P.P.M. | 5.0 | 50.0 | 5.0 | 50.0 | 50.0 |
| | | Water temperature 51° F. | | | | |
| Gills | | 29.0 | 23.3 | 31.3 | 26.4 | 37.1 |
| Digestive tract | | 3.5 | 4.5 | 3.8 | 4.5 | 4.5 |
| Remainder | | 67.5 | 72.2 | 64.9 | 69.1 | 58.4 |
| | | Water temperature 36° F. | | | | |
| Gills | | 28.1 | 22.0 | 25.3 | 28.3 | 26.8 |
| Digestive tract | | 2.3 | 3.5 | 2.3 | 2.5 | 2.1 |
| Remainder | | 69.6 | 74.5 | 72.4 | 69.2 | 71.1 |

**TABLE 4.** Distribution ratio of absorbed to total cobalt for parts of brook trout at various exposure times to labeled cobalt.

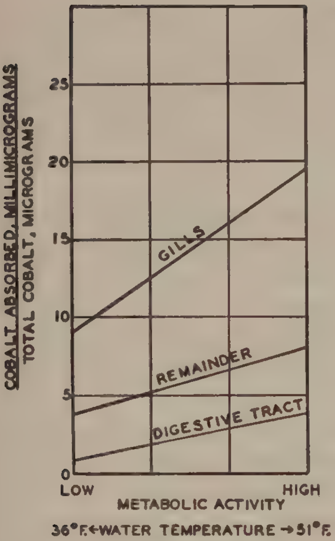
| Part | Time in hours | | | |
|---|---|---|---|---|
| | 4 | 8 | 16 | 32 |
| Gill | 2.12 | 2.60 | 2.31 | 2.38 |
| Digestive tract | 0.10 | 0.15 | 0.29 | 0.37 |
| Remainder | 1.00 | 0.91 | 0.93 | 0.90 |

tribution of absorbed cobalt of the 4 major variables. The curves represent the relationship of each of the major variables independent of the other variables of the experiment. The relative position and slopes of the curves for the 3 parts are the same for the three variables which show significant differences. The slopes of Graph 4, Chart III, are not significant. In a study of calcium absorption at this laboratory (Phillips, et al, 1954) a greater deposition of calcium from the water occurs when an abundant supply of other minerals is available from the water. Calcium is a structural material and requires other minerals for building tissues within the body.

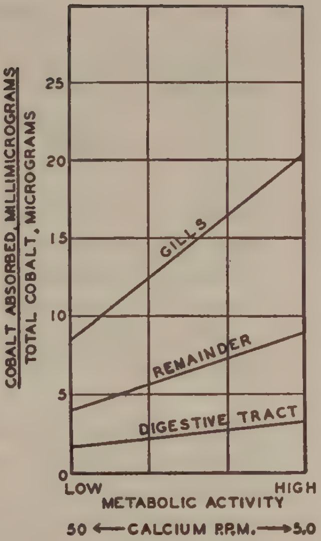
Apparently cobalt functions as a trace mineral in the trout body, similar in catalytic action to that of the vitamins (Comar, 1948), having relationships and antagonisms with other nutrients, but possessing little structural significance.

The physiological dose for a ruminant is roughly 2 millimicrograms of cobalt per gram of body weight (from the data of Comar, 1948). A maximum of 1 millimicrogram of cobalt per gram of body weight is absorbed by brook trout from the labeled water in 32 hours. The ruminant, as a result of their digestive process, requires a high level of trace elements and mineral deficiencies are more important than vitamin deficiencies. If cobalt satisfies a similar need for trout, apparently the cobalt in water supplies the cobalt requirement. This is based on the assumption that the fish have been living in cobalt-containing water for a long time and the additional uptake can occur for two reasons. First, either the cobalt is being used at a constant rate for some life process, or second, the cobalt is constantly moving in and out in an exchange process. The lack of an equilibrium appearing in any tissue in the lesser concentration of cobalt in the water and the constantly increasing distribution ratio for the gastro-intestinal tract as the time of exposure is increased in either concentration of cobalt in water, leads to the conclusion that the cobalt is being absorbed for the first reason and that the 0.25 micrograms of cobalt per liter is the more optimum concentration of cobalt.

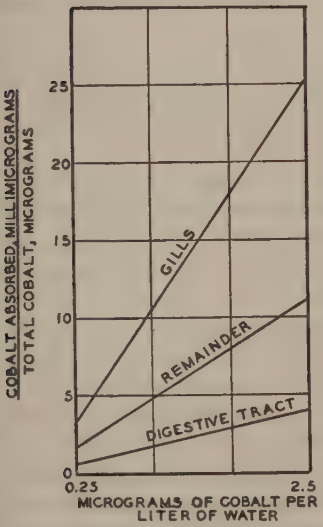
**CHART III. Graphic statistical summary of the influence of cobalt concentration, calcium concentration, temperature, and the total concentration of minerals in the water on the ratio of millimicrograms of cobalt absorbed from the water to the total micrograms contained in the gills, gastro-intestinal tract, and the remainder of brook trout.**



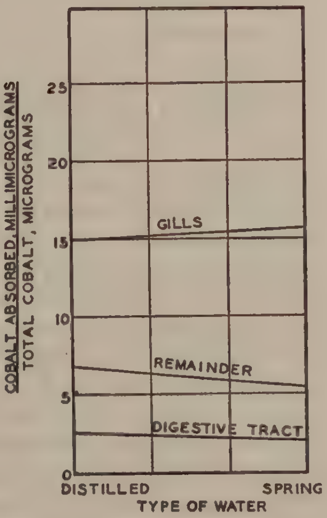
GRAPH I



GRAPH 2



GRAPH 3



GRAPH 4

## Summary

1. The concentration of cobalt in Cortland spring water (0.25 micrograms per liter) appears capable of supplying the cobalt requirement of fingerling brook trout and, compared to a 10 fold increase in cobalt concentration, is a more nearly optimum value.
2. The absorption of cobalt from water is a valid index of the metabolic activity of brook trout, increasing water temperature causing an increasing rate of absorption of the mineral. Decreasing the concentration of calcium in the water from 50 to 5 p.p.m. causes an increase in metabolic activity greater in magnitude than an increase in water temperature from 36°F. to 51°F.
3. The site of absorption of the cobalt ion is the gill. The cobalt is distributed through the body fluids, and a significant deposition of the absorbed cobalt occurs in the gastro-intestinal tract.
4. A concentration of 50 p.p.m. of calcium maintains the normal ionic regulation of brook trout. Removing all other minerals but calcium at this concentration, from normal Cortland spring water, does not cause a change in cobalt absorption. The trout's osmotic activity increases when the calcium ion concentration is lowered to 5 p.p.m. calcium in distilled water, but the osmoregulatory action of the calcium ion does not break down in this dilute media.

## Absorption of Calcium from Food and Water by Fed and Starved Brook Trout

Although trout utilize food calcium, the pioneering experiment of McCay et al (1937) proves that more calcium is found in the trout's body than can be accounted for from the calcium content of the diet of the fish. It has been experimentally simpler to conduct absorption studies at this laboratory on starving fish, ignoring the effect of feeding on the absorption of minerals from the water. Likewise, the counter effect of the absorption of minerals from the water on the absorption of minerals from the food has been by-passed.

A great advance in fisheries research is the development by Wolf (1951) of a synthetic diet for trout. This diet is used extensively by the Cortland workers in establishing the vitamin requirement for various species of trout. It is now found to be a most effective medium for labeling with radioactive tracers, allowing a quantitative control heretofore impossible with standard hatchery diets. Wolf's synthetic diet is highly homogeneous and is readily eaten by trout. There is no significant leaching of radioactivity to the water in which the fish are fed.

The level of calcium in Wolf's diet (157 milligrams per 100 grams) is low compared to a standard hatchery diet (Cortland diet, 520 milligrams per 100 grams). The levels that were established for the diet in these experiments were one half and twice that of the standard Cortland hatchery diet. The levels were:

1,040 milligrams of calcium per 100 grams for the high level diet.
260 milligrams of calcium per 100 grams for the low level diet.

These experimental diets are only relatively high and low in calcium. An analysis of natural food shows an average value of 100 milligrams per 100 grams of food (Phillips et al, 1953).

It was necessary to modify Wolf's diet to obtain the high and low levels of calcium. The radioisotope used to label the calcium when dietary calcium was traced was in the form of a solution of calcium chloride. The diets included compensation for the added chloride and it was essential not to disturb the calcium-phosphorus ratio of the diets. The chloride content of the labeled and unlabeled diets was held constant by adding calcium chloride as needed, and supplementary amounts of phosphate to maintain a 1:1 ratio of calcium to phosphorus (the ratio normally present in the diet). The ingredients were mixed as described by Wolf (1951) and the diet was fed according to standard feeding tables (Deuel et al, 1952).

Feeding trials proved this modified diet to equal Wolf's original. However, the 400 percent difference in calcium levels could cause a difference in the calcium composition of the trout body over the length of the experimental period. To test this possibility, 72 fingerling brook trout, weighing from 10 to 20 grams, which had been feeding on the standard hatchery diet, were divided into two groups of 36 trout each. One group of fish was placed in a small trough and fed the high level calcium diet for three weeks and the other group the low level calcium diet under identical conditions. After three weeks, four groups of three trout were randomly netted from each trough, killed, and dissected into parts (gills, gastro-intestinal tract, and remainder). The tract was carefully cleaned of its contents. The pooled parts of each of the three fish were analyzed for calcium following the procedure previously described (Phillips et al, 1952).

The results are summarized in Table 5. Statistical analysis shows high significance for the difference in the calcium content of the gastro-intestinal tract. The trout fed the high level calcium diet have nearly four times the amount of calcium in the gastro-intestinal tissues than is found in the same tissues of trout fed the low level calcium diet. This reflects the four times greater amount of calcium in the high level calcium diet. Although the gastro-intestinal tissues reflect the calcium content of the diet after only 3 weeks of feeding the 2 levels of calcium, this length of time is insufficient to show any significant differences in the gills or remainder of the trout. The calcium content of both of these parts tends to be slightly higher in the brook trout receiving the high level calcium diet.

## Experimental

Substituting radioactive calcium$^{45}$ for part of the calcium in the two levels of synthetic diet produced food chemically identical in terms of the calcium with the non-radioactive food, but with the calcium effectively labeled. The distribution of labeled calcium absorbed from the food was as readily traceable as was the distribution of labeled calcium from water (Phillips et al, 1954). The standard for comparison was the concentration of calcium found in the Cortland spring water (50 p.p.m.). The experimental conditions were as follows:

TABLE 5. Distribution of calcium to parts of brook trout fed high or low level calcium diets for a three week period. (Average of 12 trout).

| Measurement | Gills | Digestive tract | Remainder | Whole Fish |
|---|---|---|---|---|
| | Low level calcium diet | | | |
| Total calcium, milligrams | 6.58 | 0.14 | 99.70 | 106.42 |
| Weight of sample, grams | 0.861 | 1.118 | 22.270 | 24.249 |
| Ratio of: $\frac{\text{Total calcium, milligrams}}{\text{Weight of sample, grams}}$ | 7.642 | 0.125 | 4.477 | 4.389 |
| Percent of calcium in part | 6.2 | 0.1 | 93.7 | — |
| | High level calcium diet | | | |
| Total calcium, milligrams | 5.09 | 0.62 | 94.70 | 100.41 |
| Weight of sample, grams | 0.649 | 1.093 | 18.462 | 20.204 |
| Ratio of: $\frac{\text{Total calcium, milligrams}}{\text{Weight of sample, grams}}$ | 7.843 | 0.567 | 5.129 | 4.970 |
| Percent of calcium in part | 5.1 | 0.6 | 94.3 | — |

1. The experimental waters contained the specified concentration of calcium in Cortland spring water.
2. The two diets were the high and low calcium diets previously described. The labeled diets contained approximately 0.02 mc of calcium$^{45}$ as chloride per gram of diet.
3. Two concentrations of calcium were used in the water, 50 and 200 p.p.m., hereafter referred to as low and high calcium waters. This 1:4 ratio equalled the spread of calcium levels in the experimental diets. No confounding effect of increased metabolic activity ensued using at least 50 p.p.m. calcium as the low level concentration.
4. The periods of starvation used were one and three weeks.
5. The fish were killed either 18 hours after a meal of labeled diet or following 18 hours of immersion in labeled water. These fish were fingerling brook trout (18 to 20 grams in weight) chosen from a population of 1,000 fish taken from a hatchery raceway and held in a trough in the hatchery, where they received the standard diet before their experimental use.

## Procedure

**Effect of starvation:** For each experimental run, 36 fish were netted from the trough and placed in small holding troughs.

These fish either continued to receive the standard hatchery diet for two weeks, or were starved for the same period.

After two weeks of either feeding or starvation, 24 fish were netted from the small holding trough. These fish were separated into two groups of 12 fish each, and the groups were placed in separate conditioning aquaria.

The conditioning aquaria were 8 x 8 x 14 inches stainless steel tanks containing 8 liters of aerated, filtered water and held in the constant temperature room at 51°F. One group of 12 fish from each experimental run was conditioned in Cortland spring water with 50 p.p.m. calcium. The other group of 12 fish was conditioned in 200 p.p.m. calcium water. The fish were not fed during the conditioning period of one week. Thus, either one week or three weeks were the total periods of starvation.

At the end of one week, the fish were transferred to experimental aquaria containing concentrations of labeled calcium identical to the concentrations of calcium in the conditioning aquaria. After 18 hours of immersion in the labeled water the fish were killed. Three pooled samples of three fish from each experimental aquaria were dissected into gills, gastro-intestinal tract, and remainder. The pooled samples of the parts were analyzed for radioactivity as previously described (Phillips et al, 1954). Each experimental run was replicated at a later date to randomize the size variation of the trout.

**Effect of feeding:** Holding and conditioning procedures identical to those for the starvation studies were followed, except that either high or low level calcium diets were fed to the fish for their entire stay in both the holding troughs (two weeks) and the conditioning aquaria (one week).

After conditioning the trout in aquaria, one of two procedures was followed:

1. **The trout were fed unlabeled food and held in labeled water:** Twenty minutes after receiving their final meal, the fish were transferred to experimental aquaria containing concentrations of labeled calcium identical to the concentrations of calcium in the conditioning aquaria. After 18 hours of immersion in the labeled water the fish were killed. Sampling procedures identical to those for the starved fish were then followed. Each experiment was replicated at a later date to randomize the size variation of the fish.
2. **Trout were fed labeled food and held in unlabeled water:** The final meal of these fish was six grams of either the high level calcium diet labeled with radioactive calcium or the low level calcium diet labeled with radioactive calcium. The uneaten labeled food was carefully siphoned off after 20 minutes, and the trout were transferred to aquaria containing concentrations of unlabeled calcium identical to the concentrations in the conditioning aquaria. After 18 hours of immersion in the unlabeled water the fish were killed. Sampling procedures identical to those for the starvation fish were followed. Each experiment was replicated at a later date to randomize the size variation of the fish.

## Experimental Results

**Effect of starvation:** More calcium is absorbed from 200 p.p.m. calcium concentration in the water than from 50 p.p.m. (Table 6a). The 4:1 ratio of the water concentration is barely reflected in the ratio of absorbed calcium appearing in the gills of the fish in the two water concentrations. The gills are the site of ingress and are intimately in contact with the labeled water. The whole fish absorbs slightly less than double the amount of labeled calcium from the 400 percent greater concentration of labeled calcium in the water. Since all of these fish have been starved for either 1 or 3 weeks, the only source of calcium is the water in which they are living. A concentration of 200 p.p.m. calcium in the water apparently exceeds the level required by starving brook trout.

More calcium is absorbed from the water by fish which are starved for 1 week than by fish which are starved for 3 weeks. More absorbed calcium is distributed to the site of deposition in the bone tissues of the remainder by fish starved for 1 week than by fish starved for the longer period.

The percent of absorbed calcium distributed to the gastro-intestinal tract is constant for both periods of starvation and both concentrations of calcium in the water. The only labeled calcium which

**TABLE 6a.** Absorption of calcium, wet weight of parts, and a statistical summary of the distribution of total and absorbed calcium in starving brook trout exposed to labeled aquarium water.

| Measurement | Part | Metabolic activity | | | |
|---|---|---|---|---|---|
| | | Low<br>Starved 3 weeks | | High<br>Starved 1 week | |
| | | P.P.M. Calcium | | P.P.M. Calcium | |
| | | 50 | 200 | 50 | 200 |
| Calcium absorbed in milligrams | Gill | 0.022 | 0.088 | 0.050 | 0.096 |
| | Digestive tract | 0.002 | 0.004 | 0.003 | 0.006 |
| | Remainder | 0.158 | 0.322 | 0.280 | 0.474 |
| | Whole fish | 0.182 | 0.414 | 0.333 | 0.576 |
| Wet weight of sample, grams | Gill | 0.556 | 0.654 | 0.681 | 0.695 |
| | Digestive tract | 0.501 | 0.591 | 0.711 | 0.689 |
| | Remainder | 10.678 | 12.861 | 14.498 | 14.461 |
| | Whole fish | 11.735 | 14.106 | 15.890 | 15.845 |
| Ratio of: $\frac{\text{Absorbed calcium, milligrams}}{\text{Wet weight of sample, grams}}$ | Gill | 0.040 | 0.135 | 0.073 | 0.138 |
| | Digestive tract | 0.004 | 0.007 | 0.004 | 0.009 |
| | Remainder | 0.015 | 0.025 | 0.019 | 0.033 |
| | Whole fish | 0.016 | 0.029 | 0.021 | 0.036 |
| Ratio of: $\frac{\text{Absorbed calcium, micrograms}}{\text{Total calcium, milligrams}}$ | Gill | 5.2 | 17.6 | 9.5 | 18.0 |
| | Digestive tract | 22.5 | 39.3 | 22.5 | 50.6 |
| | Remainder | 3.3 | 5.6 | 4.2 | 7.3 |
| | Whole fish | 3.4 | 6.2 | 4.5 | 7.7 |
| Distribution of absorbed calcium (percent of absorbed calcium in the part) | Gill | 12.1 | 21.3 | 15.0 | 16.7 |
| | Digestive tract | 1.1 | 1.0 | 0.9 | 1.0 |
| | Remainder | 86.8 | 77.7 | 84.1 | 82.3 |
| Distribution of total calcium (percent of the total calcium in the part) | Gill | 5.31 | 5.31 | 5.31 | 5.31 |
| | Digestive tract | 0.33 | 0.33 | 0.33 | 0.33 |
| | Remainder | 94.36 | 94.36 | 94.36 | 94.36 |
| Distribution ratio: $\frac{\text{Percent of absorbed calcium}}{\text{Percent of total calcium}}$ | Gill | 2.28 | 4.01 | 2.82 | 3.14 |
| | Digestive tract | 3.33 | 3.03 | 2.73 | 3.03 |
| | Remainder | 0.93 | 0.82 | 0.89 | 0.87 |

the site of egress (some portion of the gastro-intestinal tract) receives is that transported from the gills to the remainder and then to the tract for elimination. Although more absorbed calcium appears in the gastro-intestinal tract of fish exposed to 200 p.p.m. calcium in water, this calcium in the tract is a reflection of the total calcium available for excretion. Consistently 1 percent of the total absorbed calcium in the trout body appears in the gastro-intestinal tract, regardless of the period of starvation or calcium concentration in the water.

The effect of starvation is constant between parts and between concentrations of calcium in the water. The decrease in absorption of labeled calcium with increased periods of starvation, appears to come from an over-all, compensating lowering of the metabolism of starving fish. These studies are not confounded by the effect of the concentration of calcium in the water on metabolic activity since the calcium content of the water is sufficient to satisfy the osmoregulatory needs (50 p.p.m.).

The distribution ratios for gill, gastro-intestinal tract, and remainder (Table 6a) parallel those of previous experiments (Phillips et al, 1954) in which brook trout are starved for only 64 hours.

### Effect of feeding:

**Trout fed unlabeled food and held in labeled water:** More calcium is absorbed by feeding fish from water containing 200 p.p.m. calcium than from water containing 50 p.p.m. calcium (Table 6b). The total amount of calcium absorbed by feeding fish in water of either concentration is greater than that absorbed by starving fish. Feeding causes a significant difference in the distribution of calcium absorbed from the water. The presence of food calcium in the gastro-intestinal tract influences the absorption of calcium from the water.

The increased metabolic activity of normal, unstarved trout causes this increased absorption of labeled calcium from the water. An additional influence is the effect of the calcium being absorbed from the food at the same time calcium is absorbed from the water.

There are no significant differences in the total absorption of calcium from the water by fish receiving either the high or low level calcium diets. However, the calcium level of the diet interacts significantly with the calcium concentration of the water and with the distribution of the calcium of the water to the parts of the fish. A combination of a high concentration of calcium in the water and a high level of dietary calcium distributes a large percentage (3.2 percent) of the absorbed calcium to the gastro-intestinal tract. As far as calcium absorbed from the water is concerned, the function of the gastro-intestinal tract is elimination. With calcium from the food supplying part of the fish's requirement for structural calcium, a large portion of the calcium absorbed from the water (the only source of labeled calcium in these experiments) is rapidly transported to the gastro-intestinal tract for elimination. A combination of high concentration of calcium in the water and a low level of dietary calcium distributes less labeled calcium (1.4 percent) to the site of egress than when a high level calcium diet is fed. The amount that is distributed is still greater than is found in starving fish (1.0 percent). When the fed fish are in water containing only 50 p.p.m. calcium, less labeled calcium is distributed to the gastro-intestinal tract (0.6 or 0.8 percent) than is distributed to the tract of starving fish. The increase in metabolism, per se, has caused more calcium from the water to be

**TABLE 6b.** Absorption of calcium, wet weight of parts, and a statistical summary of the distribution of total and absorbed calcium in feeding brook trout exposed to labeled aquarium water.

| Measurement | Part | Dietary calcium | | | |
|---|---|---|---|---|---|
| | | Low level | | High level | |
| | | P.P.M. calcium in water | | P.P.M. calcium in water | |
| | | 50 | 200 | 50 | 200 |
| Calcium absorbed in milligrams | Gills | 0.040 | 0.136 | 0.064 | 0.100 |
| | Digestive tract | 0.002 | 0.010 | 0.004 | 0.026 |
| | Remainder | 0.300 | 0.576 | 0.434 | 0.691 |
| | Whole fish | 0.342 | 0.722 | 0.502 | 0.817 |
| Wet weight of sample, grams | Gills | 0.709 | 0.869 | 0.706 | 0.755 |
| | Digestive tract | 0.858 | 0.838 | 0.877 | 0.639 |
| | Remainder | 14.166 | 16.528 | 15.055 | 15.319 |
| | Whole fish | 15.727 | 18.235 | 16.638 | 15.713 |
| Ratio of: $\frac{\text{Absorbed calcium, milligrams}}{\text{Wet weight of sample, grams}}$ | Gills | 0.057 | 0.157 | 0.091 | 0.132 |
| | Digestive tract | 0.002 | 0.012 | 0.005 | 0.041 |
| | Remainder | 0.021 | 0.035 | 0.029 | 0.045 |
| | Whole fish | 0.022 | 0.040 | 0.030 | 0.052 |
| Ratio of: $\frac{\text{Absorbed calcium, micrograms}}{\text{Total calcium, milligrams}}$ | Gills | 7.4 | 20.5 | 11.9 | 17.2 |
| | Digestive tract | 15.5 | 93.0 | 11.7 | 75.8 |
| | Remainder | 4.7 | 7.8 | 6.5 | 10.0 |
| | Whole fish | 4.7 | 8.6 | 6.4 | 11.2 |
| Distribution of absorbed calcium (percent of absorbed calcium in the part). | Gills | 11.7 | 18.8 | 12.7 | 12.2 |
| | Digestive tract | 0.6 | 1.4 | 0.8 | 3.2 |
| | Remainder | 87.7 | 79.8 | 86.5 | 84.6 |
| Distribution of total calcium (percent of the total calcium in the part). | Gills | 6.18 | 6.18 | 5.07 | 5.07 |
| | Digestive tract | 0.13 | 0.13 | 0.61 | 0.61 |
| | Remainder | 93.68 | 93.68 | 94.32 | 94.32 |
| Distribution ratio: $\frac{\text{Percent of absorbed calcium}}{\text{Percent of total calcium}}$ | Gills | 1.89 | 3.04 | 2.50 | 2.41 |
| | Digestive tract | 4.62 | 10.77 | 1.31 | 5.25 |
| | Remainder | 0.94 | 0.85 | 0.92 | 0.90 |

used by the fish. Neither of the dietary levels supplies sufficient calcium to leave an excess of labeled calcium to be distributed to the gastro-intestinal tract when the fish are held in 50 p.p.m. calcium in water. It definitely appears that calcium from the water is used for structural purposes. A combination of high water and high dietary levels of calcium is necessary for a large distribution of labeled calcium to the gastro-intestinal tract for excretion.

The distribution ratios (Table 6b) are also influenced by feeding the fish. Since an asymtotic increase to unity is found for the distribution ratio of the remainder as the time of exposure to labeled calcium in water increases (Phillips et al, 1954), the increased value of this statistic for feeding fish over starving fish indicates a more rapid rate of deposition of calcium in feeding fish. This results from the increased metabolic rate of fed fish over starving fish.

**Trout fed labeled food and held in unlabeled water**: The deposition of calcium absorbed from ingested food is studied by labeling the dietary calcium and repeating the experimental conditions previously described for labeled calcium in water. The major point of similarity between dietary and water calcium is the order of magnitude of the distribution ratio of the remainder (Table 6c). The bony tissues of the remainder are the site of deposition of calcium absorbed from the food, as was found when calcium is absorbed from water.

Since the site of absorption of the dietary calcium must be the gastro-intestinal tract, it is surprising that the same amount of labeled calcium from the food appears in the gills (on the basis of total and wet weight) as the gastro-intestinal tract (Table 6c). However, much more (12 to 70 times) absorbed calcium per gram of calcium contained appears in the gastro-intestinal tract. Great care is taken in these experiments to maintain a very high specific activity of the food calcium (millicuries per milligram of calcium) to prevent confounding if the ingested calcium were to be excreted and thus label the calcium in the water with radioactivity. No measureable labeling of the water occurs.

The maintainance of osmotic equilibrium implies the exchange of ions across the permeable surfaces of the gills. From previous work (Phillips et al, 1954) it is known that significant structural deposition of calcium occurs in the gills within 12 hours after the absorption of labeled calcium from the water, but the greater than one value of the distribution ratio for gills found in the present experiments (1.32 to 2.82) indicates that a portion of the labeled calcium from the food is transported to the gills for osmotic purposes. The labeled calcium in the gills is not from excreted labeled calcium in the water. The large storehouse of calcium in the water is unlabeled after 18 hours contact with fish that have been fed a labeled diet.

Little comment is necessary concerning the large distributions of labeled calcium from the food to the tissues of the gastro-intestinal tract. At the time of dissection, some quantity of undigested food is still present in the stomach from the meal fed 18 hours previously. A continuous absorption of calcium occurs through the walls of the gastro-intestinal tract.

Small statistical significance can be attached to the total amounts of calcium absorbed from the labeled diets. The amount of calcium absorbed by an individual trout ranges from 0.0 to 0.665 milligrams

**TABLE 6c.** **Absorption of calcium, wet weight of parts, and a statistical summary of the distribution of total and absorbed calcium in feeding brook trout fed a high or low level of labeled calcium in the diet.**

| Measurement | Part | Labeled dietary calcium | | | |
|---|---|---|---|---|---|
| | | Low level | | High level | |
| | | P.P.M. calcium in water | | P.P.M. calcium in water | |
| | | 50 | 200 | 50 | 200 |
| Calcium absorbed in milligrams | Gill | 0.008 | 0.019 | 0.005 | 0.019 |
| | Digestive tract | 0.008 | 0.020 | 0.007 | 0.021 |
| | Remainder | 0.083 | 0.151 | 0.048 | 0.093 |
| | Whole fish | 0.099 | 0.190 | 0.066 | 0.133 |
| Wet weight of sample, grams | Gill | 0.772 | 0.844 | 0.697 | 0.952 |
| | Digestive tract | 0.692 | 0.780 | 1.029 | 0.906 |
| | Remainder | 14.734 | 17.778 | 16.131 | 18.491 |
| | Whole fish | 16.198 | 19.402 | 17.857 | 20.349 |
| Ratio of: $\frac{\text{Absorbed calcium, milligrams}}{\text{Wet weight sample, grams}}$ | Gill | 0.010 | 0.023 | 0.007 | 0.020 |
| | Digestive tract | 0.012 | 0.026 | 0.007 | 0.023 |
| | Remainder | 0.006 | 0.008 | 0.003 | 0.005 |
| | Whole fish | 0.006 | 0.010 | 0.003 | 0.007 |
| Ratio of: $\frac{\text{Absorbed calcium, micrograms}}{\text{Total calcium, milligrams}}$ | Gills | 1.3 | 3.0 | 0.9 | 2.6 |
| | Digestive tract | 93.0 | 201.6 | 12.9 | 42.5 |
| | Remainder | 1.3 | 1.8 | 0.7 | 1.1 |
| | Whole fish | 1.3 | 2.1 | 0.6 | 1.5 |
| Distribution of absorbed calcium (percent of absorbed calcium in the part) | Gill | 8.1 | 10.0 | 8.3 | 14.3 |
| | Digestive tract | 8.1 | 10.5 | 11.7 | 15.8 |
| | Remainder | 83.8 | 79.5 | 80.0 | 69.9 |
| Distribution of total calcium (percent of the total calcium in the part). | Gill | 6.18 | 6.18 | 5.07 | 5.07 |
| | Digestive tract | 0.13 | 0.13 | 0.61 | 0.61 |
| | Remainder | 93.68 | 93.68 | 94.32 | 94.32 |
| Distribution ratio: $\frac{\text{Percent of absorbed calcium}}{\text{Percent of total calcium}}$ | Gill | 1.31 | 1.62 | 1.64 | 2.82 |
| | Digestive tract | 62.31 | 80.77 | 19.18 | 25.90 |
| | Remainder | 0.89 | 0.85 | 0.85 | 0.74 |

in 18 hours. However, the distributions of labeled calcium from the food parallel those distributions of labeled calcium from water, as previously discussed, e.g. more absorbed calcium remains in the stomach when more is available from the water. To completely determine the distribution of dietary calcium, a more rigid control is necessary of the amount of labeled food each trout receives. For a thorough interpretation, the gastro-intestinal tract should be separated into stomach and intestinal tissues for analyses.

Since no absolute measurement is made, little can be said about the increased absorption of calcium from the low level calcium diet by the trout. However, the acquisition of salts from the foods as well as from the water plays a part in osmotic regulation of fresh water fish and the increased absorption from a "more dilute" food follows the pattern found for calcium ions in water.

## Discussion

It is noted by Holtz et al (1947) that there is an optimum level of dietary calcium for rats. Lowering the calcium content of the diet does not cause a drop below a minimum value in the amount of calcium absorbed from the food. An excess above the optimum causes a slow drop of the calcium content of the tissues while the excretion of calcium through the intestine increases. Hansard et al (1951), Carlsson (1951), Hayami (1952), and Forunier (1953) note that rats on a low calcium diet possess an increased ability to absorb calcium$^{45}$ from the digestive tract. Experiments with trout are confounded by the dual sources available to the fish (from the water as well as the food), but the over-all picture found for calcium metabolism in brook trout follows the patterns found by other workers in the rat.

With brook trout, feeding, as such, causes an increased metabolic activity which causes more calcium to be taken from the water. The fish gradually acclimatize to starvation, decreasing the absorption of minerals from the water to a maintainance level. Feeding the fish provides protein, carbohydrate, fat, vitamins, etc. and the resulting structural growth requires additional calcium from the water supplementing the calcium in the food. Full use of the increased amount of calcium absorbed from the water, because of the increased metabolism of the fish, is not made if an excess of calcium is present in the food.

Doyle and Porter (1951) find that protein exerts an influence on bone formation when the rat is fed a low level calcium diet. The growth of soft tissues is promoted out of proportion to the growth of skeletal tissue. Sherman et al (1947) show that rats on a low calcium and high protetin diet (20 percent casein) have less calcium than rats on a low calcium and lower protein diet (14 percent). If the calcium level of the diet is increased to 640 milligrams per 100 grams as fed, no protein-calcium unbalance occurs.

If the optimum level of calcium for trout is defined as that which causes one gram of tissue to be produced with the smallest amount of calcium in the food and water, a combination of high levels in both food and water exceeds the optimum since an excess of calcium is excreted. The accumulation and turnover of calcium in the various bones of cattle parallels the metabolic activity (Hansard, 1952). Using deposition of calcium in bony tissues of the remainder of the fish as a measure, the low calcium diet fed in the 50 p.p.m. calcium water appears to produce the best calcium conversion (distribution ratio of 0.95, Table 6b). However, the small percentage (0.6 percent) of absorbed calcium appearing at the site of elimination (gastrointestinal tract) indicates a retention of calcium within the fish body. Even a starving fish eliminates more (1.0 percent) of the absorbed calcium. The high level calcium diet fed in 50 p.p.m. water appears to be slightly superior, with a distribution of 0.8 percent to the gastrointestinal tract and a distribution ratio of 0.92 for the remainder. Either diet fed to trout held in water containing 50 p.p.m. calcium produces results close to the optimum. Increasing the calcium concentration of the water to 200 p.p.m. does not produce proportionately beneficial changes if the fish is receiving dietary calcium.

Wooley and Mickelson (1954) promote increased growth and decreased mortality of rabbits by increasing the level of calcium in diets containing 30 percent casein. Less effect of increased calcium is found by Wooley and Mickelson for rabbits fed a 20 percent casein diet. It does appear that for brook trout, also, the mineral requirements are closely related to the growth promoted by all the constituents of the diet, and, specifically, to the composition of the water in which the trout lives.

## Summary

1. Starving brook trout maintain a conserving metabolism, and absorb less calcium from water than do feeding brook trout. After 3 weeks of starvation, the metabolic activity is less than after 1 week of starvation.
2. A calcium concentration of 200 p.p.m. in hatchery waters appears to exceed the requirement of either starving or feeding fish. Starving brook trout absorb less than twice as much cal-

cium when the concentration of calcium in the water increases four fold (50 to 200 p.p.m.). An excess of calcium is absorbed from the water and rapidly appears in the intestinal tract of fish feeding on calcium containing diets in waters at a concentration of 200 p.p.m. calcium.

3. Absorption of calcium from the water is not directly influenced by the level of calcium in the diet. The distribution of the absorbed calcium from different concentrations of calcium in the water to the different parts of brook trout (gills, gastro-intestinal tract, and remainder) is significantly influenced by the interaction of the dietary calcium with the water calcium. When both the water and food levels of calcium are high, a large percentage of the absorbed calcium from the water is distributed to the intestinal tract for excretion.
4. Brook trout fed a low level of calcium (260 milligrams per gram of diet) for three weeks absorb more calcium from the diet than brook trout fed a high level (1,040 milligrams per gram of diet). More of the dietary calcium appears in the gastro-intestinal tract of brook trout held in 200 p.p.m. calcium water than in the gastro-intestinal tract of trout held in water containing 50 p.p.m. calcium.
5. The amounts of calcium absorbed from water and used for structural purposes by brook trout during 18 hours of immersion in hatchery water containing calcium is at least of the same order of magnitude as that absorbed from food containing calcium during 18 hours of digestion. The calcium from the water continues to be used even though calcium from the food is made available.

## Conclusions

The over-all pattern of mineral metabolism of brook trout is confounded by the two sources of mineral nutrients to fresh-water fish. Nutrients dissolved in the water are as important as those included in the food. The osmotic regulation of trout is accomplished by selective excretion of ions in a urine more dilute in concentration than the fluids of the body and by the controlled uptake of mineral salts (Krogh, 1939). These mineral salts are acquired from the water by absorption through the fish integument and by absorption from the food through the walls of the digestive tract. The osmoregulatory effect of the calcium ion is confounded by the accompanying prime importance of calcium as a structural material.

Although cobalt (a trace element) lacks the structural significance of calcium, the physiological level of absorption of cobalt ions from the water is regulated by the fish, as well as influenced by the similar metabolic effects of increasing water temperatures and decreasing the concentration of the osmoregulatory calcium ion in the water.

The diverse functioning of minerals in the food and water is one of the primary factors of paramount importance in establishing production capacities of hatcheries or natural waters. The sum total of structural, metabolic, and osmoregulatory factors will vary for each specific water, and for each specific hatchery or natural diet.

The effect of the minerals in specific waters and diets should be determined. The study of mineral metabolism should be extended to other species of trout and to trout at different stages of growth. To correlate this knowledge with the productivity of lakes and streams, the mineral metabolism of the organisms of the natural food chain should receive attention.

## A CHEMICAL COMPARISON OF HATCHERY AND WILD BROOK TROUT

Previous experiments (Phillips et al, 1953) have shown that hatchery foods are nutritionally superior (in the chemical constituents analyzed) to the natural food of brook trout. In general, superior foods produce a superior product and therefore, a chemical comparison of hatchery and wild trout should provide a measure of the production efficiency of the two types of food. Through the cooperation of several biologists[1] during the summer and fall of 1955, a number of samples of wild brook trout, from widely separated areas of the United States and Canada, were received and chemically compared with trout reared at the Cortland Hatchery.

### Method

The wild brook trout were preserved in either 5 percent formalin or 95 percent ethyl alcohol. After drying in a vacuum oven to determine water, the samples were analyzed for protein, ash, fat, and the iodine number of the fat.[2] The wild trout averaged 14.1 grams in weight (ranging from 2.1 to 26.3 grams) and the hatchery trout 11.6.

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[1] Mr. John Appelget, Dr. Jean-Paul Cuerrier, Dr. A. H. Hazzard, Dr. Robert Lennon, Mr. Reed Nielsen, Mr. Albert Swartz, and Dr. D. A. Webster.

[2] Analytical methods: Protein, micro-Hengar; Ash, ignition to constant weight; Fat, continuous ether extraction; Iodine number of the fat, Hanus.

grams (ranging from 3.9 to 18.8 grams). Twenty-one samples of wild and 27 samples of hatchery brook trout were available for the comparisons.

The analytical data for hatchery trout represent several years' accumulation of analyses of trout fed a variety of diets.

Differences between the two groups of trout were measured by Fisher's "F" test for comparing two series of unequal numbers in which pairing of the observations is impossible. The probable error of each of the means was estimated by standard equations. Table 7 and Chart IV summarize the data.

## Chemical Comparison

**Water**: Wild trout contain significantly less water in their bodies than hatchery trout. The water content of wild trout ranges from 62.0 to 79.0 percent and the hatchery trout from 71.0 to 82.0 percent.

**Protein**: The average protein content of wild trout (21.2 percent) is significantly higher than that of hatchery trout (13.7 percent). The range for wild trout is from 16.0 to 25.3 percent and from 10.1 to 15.2 percent for hatchery trout.

**Ash**: The average percent ash in the body of wild trout is higher (3.3 percent) than that of hatchery trout (2.0 percent). The results range from 2.3 to 5.4 for wild and from 1.7 to 2.4 for hatchery trout.

**Fat**: There is significantly less fat in the body of wild trout (3.4 percent) than in the body of hatchery trout (5.5 percent). The analyses range from 1.2 to 6.3 for wild trout and from 3.1 to 7.3 for hatchery trout.

**Iodine number of the fat**: The iodine number of the fat of wild trout is higher (135) than that of hatchery trout (100). The values for wild trout range from 111 to 171 and for hatchery trout from 89 to 111.

**Probable error of the means**: All of the means listed in Table 7 are good estimates of the averages as proven by the probable error of the means.

**Effect of trout size**: Since the wild and hatchery trout are similar in size (ranging from 2.1 to 26.3 for wild and 3.9 to 18.8 for

TABLE 7. A chemical comparison of hatchery and wild brook trout.

| Sample number | Average weight grams | | Percent water | | Percent protein | | Percent ash | | Percent fat | | Fat iodine number | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Wild | Hatchery | Wild | Hatchery | Wild | Hatchery | Wild | Hatchery | Wild | Hatchery | Wild | Hatchery |
| 1 | 4.3 | 10.0 | 72.0 | 77.0 | 20.0 | 14.4 | 3.7 | 2.3 | 2.0 | 5.0 | 171 | 95 |
| 2 | 20.9 | 10.2 | 62.0 | 78.0 | 25.3 | 13.8 | 3.7 | 1.9 | 6.2 | 5.2 | 142 | 95 |
| 3 | 6.2 | 13.0 | 71.0 | 76.0 | 21.1 | 14.2 | 4.2 | 1.9 | 2.0 | 6.5 | 168 | 102 |
| 4 | 8.6 | 13.7 | 71.0 | 76.0 | 19.4 | 13.9 | 4.2 | 1.9 | 3.7 | 6.8 | 136 | 107 |
| 5 | 9.7 | 9.3 | 67.0 | 76.0 | 25.2 | 14.1 | 3.7 | 2.0 | 4.8 | 5.3 | 143 | 93 |
| 6 | 26.3 | 10.4 | 68.0 | 77.0 | 23.4 | 13.6 | 5.4 | 1.9 | 3.5 | 5.1 | 137 | 93 |
| 7 | 13.9 | 14.6 | 67.0 | 71.0 | 24.1 | 14.5 | 2.5 | 2.2 | 3.8 | 7.3 | 141 | 111 |
| 8 | 16.8 | 14.0 | 67.0 | 76.0 | 25.2 | 14.5 | 2.9 | 1.8 | 3.8 | 7.2 | 150 | 110 |
| 9 | 19.8 | 10.7 | 66.0 | 78.0 | 24.6 | 14.1 | 3.6 | 2.0 | 4.7 | 3.8 | 136 | 92 |
| 10 | 25.8 | 9.5 | 69.0 | 80.0 | 23.4 | 13.7 | 2.6 | 1.7 | 4.3 | 3.1 | 131 | 93 |
| 11 | 18.3 | 4.7 | 78.0 | 79.0 | 17.0 | 12.5 | 3.1 | 2.0 | 1.2 | 4.2 | 129 | 91 |
| 12 | 26.0 | 8.1 | 77.0 | 76.0 | 17.0 | 13.6 | 2.6 | 2.4 | 3.2 | 5.6 | 111 | 95 |
| 13 | 16.1 | 3.9 | 79.0 | 82.0 | 16.0 | 11.2 | 2.3 | 1.7 | 1.8 | 5.6 | 113 | 92 |
| 14 | 16.1 | 10.6 | 76.0 | 82.0 | 17.8 | 10.1 | 3.0 | 2.1 | 1.6 | 5.2 | 129 | 89 |
| 15 | 23.3 | 10.0 | 79.0 | 76.0 | 16.5 | 14.8 | 2.6 | 2.2 | 1.2 | 5.5 | 125 | 107 |
| 16 | 7.2 | 9.4 | 79.0 | 78.0 | 16.2 | 14.3 | 2.4 | 1.9 | 1.8 | 5.3 | 131 | 103 |
| 17 | 25.6 | 6.3 | 74.0 | 79.0 | 20.2 | 13.8 | 2.7 | 2.2 | 3.1 | 3.8 | 112 | 89 |
| 18 | 2.1 | 11.6 | 70.0 | 79.0 | 21.0 | 12.8 | 3.7 | 2.2 | 6.3 | 3.9 | 144 | 91 |
| 19 | 3.0 | 8.9 | 72.0 | 78.0 | 21.6 | 13.0 | 3.3 | 2.2 | 3.4 | 6.1 | 145 | 110 |
| 20 | 2.9 | 15.6 | 67.0 | 75.0 | 25.3 | 14.1 | 3.7 | 1.9 | 4.6 | 6.9 | 126 | 103 |
| 21 | 3.2 | 15.4 | 69.0 | 75.0 | 22.5 | 14.2 | 3.0 | 2.0 | 4.6 | 6.7 | 117 | 103 |
| 22 | — | 14.2 | — | 76.0 | — | 14.1 | — | 1.8 | — | 6.3 | — | 109 |
| 23 | — | 15.6 | — | 75.0 | — | 14.2 | — | 2.0 | — | 6.7 | — | 106 |
| 24 | — | 17.3 | — | 78.0 | — | 15.2 | — | 1.8 | — | 4.8 | — | 106 |
| 25 | — | 14.2 | — | 79.0 | — | 13.6 | — | 1.9 | — | 4.7 | — | 104 |
| 26 | — | 13.7 | — | 76.0 | — | 14.6 | — | 2.0 | — | 5.5 | — | 102 |
| 27 | — | 18.8 | — | 77.0 | — | 14.3 | — | 1.9 | — | 5.9 | — | 110 |
| Average | 14.1 | 11.6 | 71.5 | 77.2 | 21.2 | 13.7 | 3.3 | 2.0 | 3.4 | 5.5 | 135 | 100 |
| "t" | | | 5.27 | | 10.68 | | 8.4 | | 5.34 | | 9.9 | |
| P.E. mean | | | ±0.73 | ±0.30 | ±0.48 | ±0.14 | ±0.11 | ±0.03 | ±0.22 | ±0.14 | ±2.23 | ±0.97 |

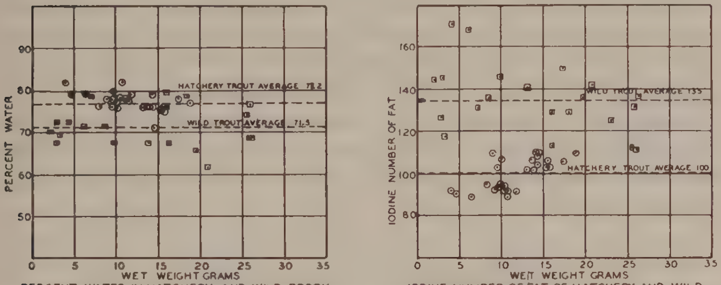
hatchery trout) the differences that are found between the two groups are not considered a function of the size of the trout. Within each group there is no correlation between the size of the trout and the chemical content of the body for water, protein, ash, or the iodine number of the fat. The plotting of the data (Chart IV) does indicate that in hatchery trout there is an increase in body fat as the trout become larger. This correlation does not exist in wild trout. These conclusions apply to trout of the size range of these experiments. It is possible that a greater range in trout size would show correlations between trout size and the chemical content of the bodies of both groups.

## Discussion

On the basis of these analyses, wild trout are chemically superior to hatchery trout. The protein and ash contents are higher in wild trout, indicating the production of a greater amount of true fish flesh from natural than from hatchery foods. The fat and water contents of hatchery trout are higher than those of wild trout. Neither fat nor water represent true fish flesh production. In hatchery trout, the relatively low level of protein and high level of fat suggest that some of the dietary protein is metabolized to body fat and is not utilized for the primary purpose of protein within the body (flesh production). The body fat of wild trout is more unsaturated than that of hatchery trout (iodine number of the fat). These differences are correlated with similar differences in the iodine number of the fats in the two types of food. The hydrogenation of unsaturated fats of goldfish diets lowers the resistance of the fish to both high and low water temperatures (Hoar and Cottle, 1952). It is open to speculation whether the relatively saturated fats in the bodies of hatchery trout will have a similar effect on their resistance to extremes of water temperature.

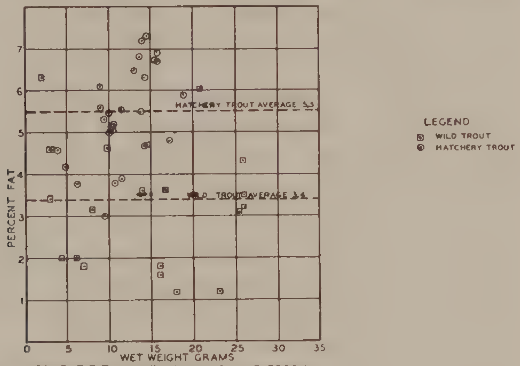
The range of analytical results is greater in wild trout than in hatchery trout (Chart IV). The water content of the body has a range difference of 17 in wild and 11 in hatchery; the protein 9.3 in wild and 5.1 in hatchery; ash 3.1 in wild and 0.7 in hatchery; and the iodine number of the fat 60 in wild and 22 in hatchery trout. The range in fat content is similar for both groups of trout (5.1 for wild and 4.2 for hatchery). A variety of feeding habits is expected among wild trout since the food consumed depends on the organisms present in the environments. In hatcheries the food is somewhat standardized and, although there is a difference in diet mixtures, the variation in chemical composition of natural foods does not occur in the majority of hatchery diets. The range of chemical composition of the

**CHART IV.** Graphical summary of the chemical composition of hatchery and wild brook trout.

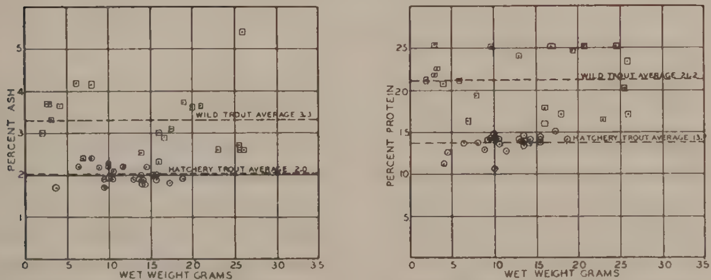


PERCENT WATER IN HATCHERY AND WILD BROOK TROUT

IODINE NUMBER OF FAT OF HATCHERY AND WILD BROOK TROUT



PERCENT FAT IN HATCHERY AND WILD BROOK TROUT



PERCENT ASH IN HATCHERY AND WILD BROOK TROUT

PERCENT PROTEIN IN HATCHERY AND WILD BROOK TROUT

wild trout doubtlessly reflects a difference in foods available to the different samples of wild trout from the environments represented in these studies. Less spreading of the data is found in plotting the chemistry of hatchery trout than in the chemistry of wild trout (Chart IV).

Since the number of samples of wild trout are few, any conclusions must be restricted to a comparison of these analyses and it is not possible to generalize. However, the samples of wild trout are from widely separated areas and do reflect a variety of conditions as evidenced by the greater probable errors of the averages of the wild trout samples. Even though the probable errors of the averages are two to three times greater for all analytical categories of wild trout, the comparison of these means with hatchery trout show highly significant differences.

## Food Sources and Body Chemistry

On the basis of these analyses of the bodies of wild and hatchery trout, interesting speculative comparisons of the effect of food sources on body chemistry are inferred. Although Phillips et al (1954) state that hatchery foods are nutritionally superior (in items tested) to natural foods, it is concluded from these experiments that natural foods do produce trout, in many chemical respects, superior to trout reared on hatchery foods.

Comparisons of the chemical composition of natural and hatchery foods emphasize some outstanding differences. Table 8 lists a typical hatchery diet with the chemical contributions of each ingredient. Table 9 lists the chemical composition of seven organisms representing the natural food of trout.

Hatchery foods contain approximately half the water, twice the protein, five times the ash, and slightly more fat than natural foods. The iodine number of the hatchery foods is less than the iodine number of the fat of natural foods.

In the body of wild trout, natural food, with half the protein content of hatchery food, produces a higher level of body protein than is found in hatchery fish; with one fifth the ash, a greater level of body ash; and with twice the water content a significantly lower level of body water. The fat content of hatchery fish is higher than is justified by the difference in fat content of the two types of food. The higher level of body fat in hatchery trout suggests the utilization of some of the food protein for fat formation (since the two food fats are within close range). This conclusion is substantiated in part by the poor utilization of starches by trout (Phillips et al, 1948), thus eliminating the starches of hatchery diets in part as a major contributing factor in fat formation. The possible formation of fat from protein in hatchery diets indicates a less efficient utilization of hatchery food than of natural food for flesh production.

The conversion of foods into fish flesh is the best measure of the efficiency of food utilization. A conversion of from 3 to 4 pounds of hatchery food to one pound of trout produced is considered satisfactory in trout hatchery management (Deuel et al, 1952). Proper feeding methods and diets duplicate these conversions under a variety of hatchery conditions. The literature reports inconsistent results for the conversion of natural foods into fish flesh. Surber (1935) reports a conversion of 6.6; Pentelow (1939) a conversion of 5.0; and Schaeperclaus (1933) a conversion of 3.9 in rearing trout on the crustacean *Gammarus*. Thus, a variety of results are reported for the same food organism. With other natural foods Schaeperclaus (1933) finds a conversion of 4.4 when feeding *Chironomus* (midge fly); Cornelius (1933) 2.3 when feeding trout (cannibalism); and the Cortland workers (unpublished) 7.1 when feeding the maggots of the house fly to trout. Recent experiments at the Cortland hatchery (unpublished) have resulted in a conversion of 2.7 when rearing brook trout on brine shrimp. In experiments in which high conversions are reported, underfeeding of the trout is evident in the experimental design. Because of the lack of agreement in the published experimental results, it cannot be concluded that natural foods are less efficient than hatchery foods in the pounds of food required to produce one pound of trout.

Since the measure of true growth is the production of fish flesh,

**TABLE 8.** Contributions of various ingredients to the chemical composition of 100 pounds of a hatchery diet.

| Ingredient | Total pounds present | Pounds per 100 pounds of diet as | | | | | Iodine number of fat |
|---|---|---|---|---|---|---|---|
| | | Protein | Ash | Fat | Water | Carbohydrate | |
| Beef spleen | 30 | 5.4 | 0.4 | 0.7 | 22.6 | 0.0 | — |
| Beef liver | 20 | 4.0 | 0.3 | 0.6 | 14.5 | 0.5 | — |
| Fish meal | 12 | 8.2 | 1.3 | 0.2 | 1.2 | 0.6 | — |
| Cottonseed meal | 12 | 4.6 | 0.8 | 1.0 | 1.2 | 4.8 | — |
| Dried skim milk | 12 | 4.2 | 1.0 | 0.1 | 1.2 | 6.0 | — |
| Wheat middlings | 12 | 2.1 | 0.5 | 0.6 | 1.2 | 7.5 | — |
| Salt | 2 | 0.0 | 2.0 | 0.0 | 0.0 | 0.0 | — |
| Total pounds or percent | 100 | 28.5 | 6.3 | 3.2 | 41.4 | 19.4 | 89 |

a comparison of the efficiency of hatchery and natural foods is possible by establishing a protein conversion for each. This is accomplished by converting the total conversion (pounds of food necessary to produce one pound of trout) to terms of food and body protein.

Experiments and experience over a number of years have established a total conversion of between 3.0 and 4.0 as a fair value for the usual type of hatchery foods (Deuel et al, 1952). Using a value of 3, the calculations for hatchery diets are:

100 pounds of food provide 28.5 pounds of protein (percent protein in the hatchery diet).

100 pounds of food produce 33.3 pounds of trout (conversion of 3).

33.3 pounds of trout produced X 13.7 (percent protein in hatchery trout) = 4.6 pounds of body protein produced.

28.5 (pounds protein fed) ÷ 4.6 (pounds protein produced) = *6.2 pounds* of food protein required to produce one pound of body protein.

Although from the literature the average conversion for natural food is 4.9 (6 experiments with a range of 2.3 to 7.1) under experimental conditions, to place a bias against natural food, a total of 6.0 pounds of natural food per pound of wild trout produced is assumed. The calculations are:

**TABLE 9.** Chemical composition of some natural fish food.

| Organism | Percent water | Percent protein | Percent ash | Percent fat | Iodine number of fat |
|---|---|---|---|---|---|
| Ephemerida (*Baetis*) | 87.2 | 8.2 | 1.0 | 1.6 | 106 |
| Plecoptera (*Acroneuria*) | 76.5 | 17.9 | 1.1 | 3.2 | 130 |
| Plecoptera (*Pteronarcys*) | 72.5 | 15.8 | 1.9 | 6.1 | 133 |
| Trichoptera (*Brachycentrus*) | 87.3 | 8.4 | 0.8 | 1.8 | 123 |
| Diptera (*Simulium*) | 86.5 | 9.0 | 2.3 | 0.7 | 131 |
| Amphipoda (*Gammarus*) | 87.5 | 6.4 | 2.7 | 0.8 | 119 |
| Oligochaeta (*Helodrilus*) | 77.8 | 14.8 | 2.0 | 1.4 | 131 |
| Average values | 82.2 | 11.5 | 2.7 | 2.2 | 125 |

100 pounds of natural food provide 11.5 pounds of protein (percent protein in natural food)

100 pounds of natural food produces 16.7 pounds of wild trout (conversion of 6.0)

16.7 (pounds of wild trout produced) X 21.2 (percent protein in wild trout) = 3.5 pounds of body protein produced.

11.5 (pounds natural food protein fed) ÷ 3.5 (pounds of body protein produced) = *3.3 pounds* of natural food protein required to produce one pound of body protein.

Thus assuming a total conversion of 6.0 for natural food and 3.0 for hatchery food, the efficiency of natural food in terms of body protein produced is almost twice that of hatchery food.

Although management practices establish the protein level in trout hatchery diets containing dry concentrates at between 28 and 30 percent, there is belief that such levels are higher than necessary. The accepted dietary level for hatchery foods is based upon the protein content of diets producing satisfactory growth rates and total conversions. The protein required in other types of animal husbandries ranges from 14 to 22 percent of the diet. Invariably trout diets composed of meats and dry concentrates and containing such low levels of protein produce inferior growth and increase the total conversion. That trout can grow efficiently on low level protein foods seems established by the production efficiency of natural foods which contain only 11.5 percent protein. There are several possible reasons for the established high protein content of hatchery diets. Hatchery diets may lack unknown vitamins necessary for growth and protein utilization. The protein quality of natural foods may be superior to that of hatchery foods, accounting for the more efficient utilization of protein in natural foods. On the other hand, the protein quality of hatchery diets may be equal to that of natural foods, but during the mechanics of feeding some of the protein may be lost because of leaching of the more soluble fractions of protein. Such losses could lower the protein quality of the diet and reduce efficiency, even though before feeding the diet is adequate in every respect. None of the analyses of hatchery trout pertain to trout fed a pelleted type of fish food. It is probable that less food is lost in feeding pellets and thus the prevention of leaching of dietary ingredients would increase the efficiency of trout flesh production. This is supported in part by the low conversions of food into fish flesh (2.0 to 2.5) when pellets are fed to trout. Pelleted types of hatchery foods offer an opportunity for reducing the protein level in the food and (since protein is the most expensive ingredient) lowering the cost per pound of hatchery diets.

It is possible that natural food is more completely absorbed than hatchery food because of more efficient digestion and thus a greater portion of natural food is utilized.

In natural habitats there are foods available to trout that approach hatchery diets in chemical content (other fish for example) but it has been reported by many workers that the types of invertebrates represented in the averages of Table 9, form from 90 to 99 percent of the food eaten by brook trout under 6 inches in length, within the size range of the brook trout used in these studies (Juday, 1905; Clemens, 1928; Needham, 1930; Lord, 1933). The comparisons between the two groups of foods are considered valid.

These comparisons of the effect of food sources on the chemical composition of the body of trout must be considered speculative for several reasons. Although the size of the wild fish is known, the age of the trout is unknown, and age might be a contributing factor. In addition, hatchery trout are fed a specified amount of food daily whereas the intake of wild trout is not controlled. However, in view of the fat content of the two groups of trout (3.4 percent for wild and 5.5 for hatchery) it is doubtful if underfeeding is a factor in the results found with wild trout. Exercise could be a most important cause of variance and undoubtedly wild trout receive more exercise than do hatchery trout. However, some of the samples used in these studies were from lakes and there is no outstanding differences between these trout and those from streams. Trout from lakes would presumably receive exercise similar to that of hatchery trout in ponds.

The analytical results certainly point toward the superiority of natural food over hatchery diets even though the natural food was not "fed" under precise experimental conditions. After years of study the rations now fed to domesticated animals are considered superior to the "natural" food of these animals. It is reasonable to expect that as research on trout nutrition progresses, the time will come when the food of trout in hatcheries will be superior in all respects to natural food. To accomplish this it will be necessary to establish conversion factors for natural foods under precise experimental conditions to verify the efficiency of these foods. Future studies must then determine the factors present in natural foods, and absent in hatchery diets, that result in the apparent superior performance of natural foods in producing trout flesh.

# THE CHEMISTRY OF DEVELOPING BROWN TROUT EGGS

The chemical embryology of developing trout eggs has received little attention. Since such studies have proven invaluable in other husbandries in establishing nutritional and physiological requirements of the female animal, a study has been initiated to determine the chemistry of developing trout eggs. Hayes (1930, 1930a) reports that the wet weight of salmon eggs increases during incubation; the protein, after an initial decrease, increases; and the fat content first increases and then decreases as the hatching period approaches. Hayes' work is based on the later stages of development after the embryo is well formed. Lovern (1934, 1936) states that the fat content of the salmon egg is a reflection of the fat within the mother's body and the characteristics of the egg fat change as the egg develops. He reports that the iodine number of the egg fat shifts from a high of 206 to a low of 176 just before hatching. Very little work has been reported on trout eggs although there is common belief among fisheries workers that the wet weight of trout eggs increases as development progresses.

To determine the chemical changes that occur in brown trout eggs during incubation, a series of samples were taken from a single lot of eggs, at weekly intervals, from the time of water hardening through the hatching period. The samples consisted of two groups of 300 eggs, which were weighed before preserving in 95 percent ethyl alcohol. After drying to constant weight to determine moisture, the samples were analyzed for protein, ash, fat, and the iodine number of the fat.[1]

Because no other adults were available, the eggs for these studies were taken from three-year-old brown trout subjected to light control to induce early spawning (spawning occurred approximately one month earlier than normal).

Table 10 and Chart V summarize the results. All data are averages of duplicate analyses upon duplicate samples. The eggs were in the eyed stage by the fourth week, in the process of hatching the sixth week and in the seventh week the samples consisted of week old fry.

## Chemical Changes

**Water:** The percent water in the developing egg decreases with time until the end of the fifth week, after which the water content increases rapidly. The newly hatched fry have considerably more water than do water hardened eggs.

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[1] Analytical methods: Protein, micro-Hengar; Ash, ignition to constant weight; Fat, continuous ether extraction; Iodine number of the fat, Hanus.

**Protein:** The protein content of the egg increases gradually to the third week, then decreases throughout the remainder of the incubation period. The newly hatched fry contain the lowest level of protein found in these studies.

**Ash:** The ash content of both eggs and newly hatched fry is constant throughout the period covered by these analyses.

**Fat:** The fat content of the egg decreases the first four weeks and then increases for the remainder of the experimental period.

**Iodine number of the fat:** The iodine number of the fat does not change throughout the experimental period.

**Wet weight of eggs:** There is a gradual decrease in the wet weight of the eggs during the first three weeks of incubation, followed by three weeks of constant weight. After hatching, the week old fry show a marked increase in wet weight.

## Discussion

The initial increase in percent protein of the egg may be correlated with the decrease in percent water. The relatively constant level of the protein on a dry basis during this period supports this correlation (Table 10). The initial decrease in percent fat indicates that fat is burned to meet the energy requirements of the developing embryo. After the third week the catabolism of the eggs has apparently shifted. The protein decrease and the fat increase indicate a surplus of protein, in so far as tissue development is concerned. The excess protein may be metabolized to fat within the embyro. The same trends in protein and fat percents of the egg are apparent in the analytical results expressed on a dry basis (Table 10). Under these circumstances the changes in fat and protein of the egg cannot be correlated with the increase in percent water. It is surprising that the percent ash remains constant throughout incubation. A mineral uptake from the water has been shown in developing salmon eggs. Hayes et al (1946) reports that there is an increase in the calcium content of salmon embryos that cannot be accounted for by the calcium present in the yolk of the egg. If a mineral uptake occurs in these studies, it must be accompanied by a similar loss since the total ash fails to show any changes throughout the period of development.

**TABLE 10.** Chemical analyses of developing brown trout eggs (weekly intervals)[1].

| Time in weeks | Total wet weight sample grams | Dry Basis | | | Wet Basis | | | | Iodine number of fat |
|---|---|---|---|---|---|---|---|---|---|
| | | Percent protein | Percent ash | Percent fat | Percent water | Percent protein | Percent ash | Percent fat | |
| 0 | 17.3 | 72.3 | 3.87 | 11.7 | 68.1 | 23.1 | 1.23 | 3.7 | 134 |
| 1 | 17.1 | 74.4 | 3.84 | 10.3 | 67.7 | 24.0 | 1.14 | 3.4 | 148 |
| 2 | 16.1 | 73.8 | 3.83 | 9.2 | 66.4 | 24.7 | 1.29 | 3.1 | 139 |
| 3 | 16.1 | 72.6 | 3.75 | 7.9 | 65.8 | 24.8 | 1.28 | 2.7 | 157 |
| 4 (eyed eggs) | 15.9 | 74.3 | 4.08 | 7.6 | 67.3 | 24.2 | 1.43 | 2.5 | 152 |
| 5 | 15.9 | 67.8 | 3.63 | 9.0 | 64.0 | 23.9 | 1.29 | 3.1 | 138 |
| 6 (hatching) | 15.9 | 70.3 | 3.88 | 10.9 | 68.3 | 22.3 | 1.23 | 3.4 | 137 |
| 7 (sac fry) | 20.1 | 68.5 | 4.40 | 12.7 | 73.9 | 17.3 | 1.14 | 3.3 | 150 |

[1] All values are averages of duplicate analyses on duplicate samples of 300 eggs each.

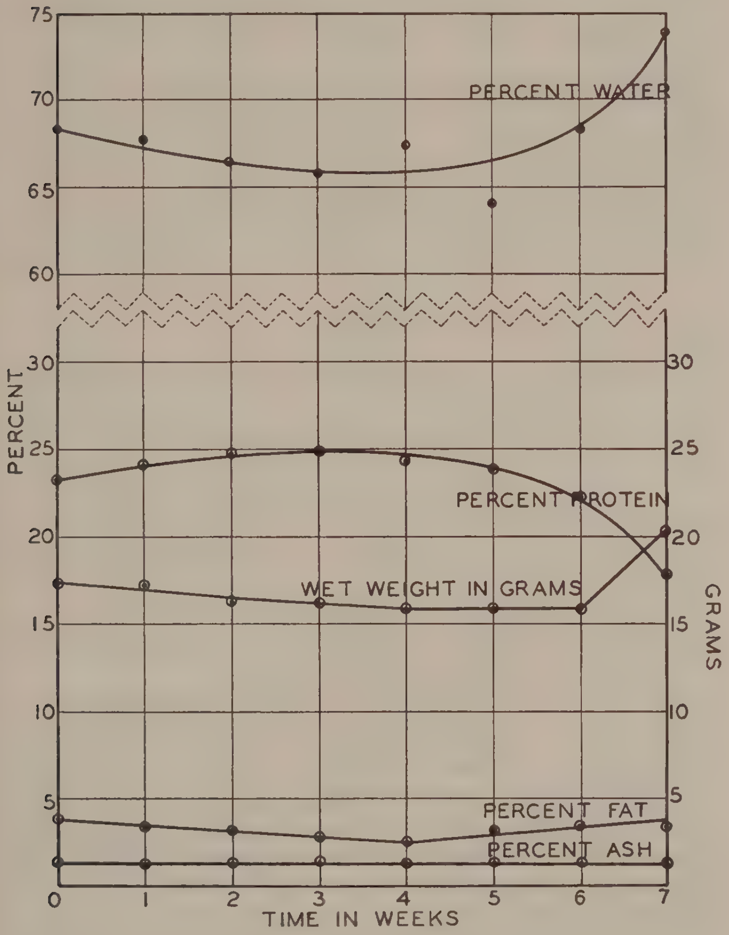
In Chart V all of the plotted points fit the hand drawn curves with the exception of the curve for percent water. The amounts of water within the egg at the fourth and fifth week do not fall on the curve that is drawn. This appears to be an analytical error, but duplicate analyses upon the two samples of eggs (300 eggs each sample) are in reasonable agreement (67.0 and 66.7 percent for the fourth week and 63.3 and 65.8 the fifth week). The possibility exists that these two points are as plotted and they reflect the previously hypothesized change in catabolism of the developing egg. The shift in protein and fat content of the developing egg occurs at about the same time interval (fourth and fifth week). This indicates the maximum need of the embyro for protein occurs at this stage of developmemnt. The excess protein may be deposited as fat and/or burned for energy, both processes forming nitrogenous wastes, accounting for the increase in water to dilute the waste products, followed by a decrease in water after equilibrium is established.

Some of these observations are not in agreement with accepted beliefs of published reports. The wet weight of brown trout eggs decreases to a constant level during incubation instead of increasing, as is commonly believed, and as Hayes (1930) reports in his study on salmon eggs. The data in Hayes' report do not agree with his conclusions. The wet weight of the entire egg does not increase significantly but the wet weight of the embyro does. It seems likely that an error has caused the replacement of the word "embyro" with the word "egg". The changes in both fat and protein content of the eggs during incubation in the present studies are the opposite of the conclusions of Hayes (1930a). These deviations are difficult to explain except through the possibility of species difference or changes brought about by the light control of the adult trout to induce early spawning. Hayes worked with wild salmon and the present studies deal with hatchery trout. Whether or not the diet of wild salmon (natural food) and the diet of hatchery trout (hatchery food) has an effect is unknown. Since Hayes worked only with the later stages of development, it is possible that different conclusions would result if the entire period of incubation had been studied.

An interesting implication of these studies is the change in metabolism of the egg during incubation and the possible effect the diet of the mother exerts upon the storage of material within the egg, thus influencing the normal metabolism of development.

These studies should be expanded to include the effects of diet of the adults, physical environment, chemical environment, and species on the chemistry of developing brown trout eggs and sac fry.

CHART V. Graphical summary of the chemical analyses of developing brown trout eggs.



## THE VITAMIN REQUIREMENT OF BROWN TROUT

Until recent years, a lack of knowledge of the vitamin requirement of trout has limited the formulation of hatchery diets. Wolf (1951) has developed a synthetic diet making possible qualitative studies on the vitamin requirement of trout. Using this diet, the same author has established the vitamin requirement for rainbow trout (1951). Phillips et al, 1952, 1953) have determined the need of brook trout for seven members of the vitamin B complex. Brown trout are one of the commonly reared species in trout hatcheries but only one preliminary report has appeared on the vitamin requirement of these fish (Phillips et al, 1954).

For the estimation of the need of brown trout for several members of the vitamin B complex, a series of vitamin omission studies were completed utilizing Wolf's synthetic diet. The trout were held over a 20 week experimental period in troughs supplied with water at an average temperature of 51°F. The handling of the experimental trout followed the method of Tunision et al (1939). Significant differences were measured by analysis of variance.

## Discussion

From the data in Table 11 all vitamins omitted from the synthetic diet are necessary growth factors for brown trout (inositol, $B_{12}$, niacin, pyridoxine, choline, riboflavin, folic acid, and biotin).

Only a deficiency of three of the vitamins results in an increase in mortality of the trout. The absence of pyridoxine from the diet causes the death of all trout within 14 weeks. The removal of either riboflavin or biotin causes an increase in trout loss but less severe than the omission of pyridoxine.

Blood counts fail to show anemia in any of the trout in these studies. Similar results are reported for brook trout (Phillips et al, 1952, 1953) and for rainbow trout (Wolf, 1951).

In comparison to the control diet, the complete synthetic diet produces satisfactory results with brown trout in this type of study.

Although eight members of the vitamin B complex are needed for normal growth and to prevent mortality of brown trout, only a deficiency of biotin results in recognizable deficiency symptoms. "Blue-slime" or "slime-patch" disease is apparent on brown trout lacking biotin in the diet. Since a lack of seven other vitamins affects the growth of brown trout, and since no symptom is recognizable to indicate which of the vitamins may be concerned, it is necessary to include all of the vitamins in all diets to assure normal growth and to prevent excessive mortalities of the trout. Experience indicates that either 5 percent dried brewer's yeast or 20 percent beef liver in the diet will satisfy the requirement of the trout for these members of the Vitamin B complex.

Future studies should include an investigation of the vitamin B requirement for both lake trout and Atlantic salmon. The completion of these studies would provide knowledge of the vitamin B requirement for all of the cold water species of fresh water fish cultured on the east coast.

**TABLE 11.** **Experiments upon the vitamin B complex requirement of brown trout over a 20 week experimental period.**

| Diet number | Vitamin omitted[1] | Average weight at end in grams[2] | Total gain in percent | Total conversion | Average loss per 2 week period in percent |
|---|---|---|---|---|---|
| 1497 | None | 9.26 | 494 | 3.9 | 0.9 |
| 1498 | None | 10.30 | 560 | 3.8 | 0.8 |
| 1499 | Control | 9.78 | 525 | 3.9 | 1.2 |
| 1500 | Control | 9.61 | 515 | 4.1 | 1.2 |
| 1501 | Inositol | 7.44 | 376 | 4.2 | 0.9 |
| 1502 | Inositol | 8.19 | 425 | 4.1 | 1.3 |
| 1503 | Vitamin $B_{12}$ | 8.50 | 445 | 3.9 | 0.9 |
| 1504 | Vitamin $B_{12}$ | 8.31 | 432 | 4.1 | 0.6 |
| 1505 | Niacin | 7.55 | 384 | 4.4 | 1.1 |
| 1506 | Niacin | 6.20 | 297 | 5.2 | 0.7 |
| 1507 | Pyridoxine | 3.15 | 102 | Complete loss end of 14 weeks | |
| 1508 | Pyridoxine | 2.46 | 58 | | |
| 1509 | Choline | 5.80 | 272 | 5.3 | 1.1 |
| 1510 | Choline | 5.56 | 256 | 5.4 | 1.1 |
| 1511 | Riboflavin | 5.75 | 268 | 5.5 | 5.2 |
| 1512 | Riboflavin | 6.94 | 345 | 4.5 | 3.1 |
| 1513 | Folic acid | 5.94 | 280 | 5.5 | 1.5 |
| 1514 | Folic acid | 5.58 | 258 | 5.8 | 1.1 |
| 1515 | Biotin | 4.71 | 202 | 6.8 | 5.7 |
| 1516 | Biotin | 4.34 | 178 | 6.1 | 4.0 |

[1] All trout fed the synthetic diet of Wolf, 1951, minus the vitamin indicated, except the control lots which received a diet composed of 50 percent beef melts and 50 percent No. 6 dry mixture. No. 6 dry mixture composed of 21.5 parts each of cottonseed meal, wheat middlings, fish meal and dried skim milk, 10 parts dried brewer's yeast and 4 parts of salt.

[2] Average weight at start 1.56 grams.

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# The Accumulation and Exchange of Strontium by Marine Planktonic Algae[1]

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## ABSTRACT

The radioactivity taken up by each of twelve species of algae, grown in a mixture of radioactive strontium and yttrium, was separated into that due to each isotope. This was accomplished by following the decay curve of the activity in the algae. The uptake of radioactive strontium and yttrium was followed at frequent intervals in *Carteria* cells grown in medium containing these two isotopes. The removal of yttrium from the medium by *Carteria* was shown to be due to adsorption on the surface, whereas strontium was taken into the cells.

It was found that *Carteria* could not divide in the absence of calcium in the medium. Neither could strontium be substituted for calcium in the metabolism of *Carteria* cells. When other factors were kept constant, the amount of strontium removed by the cells was dependent upon the strontium concentration in the medium.

With the radioisotopic dilution technique, the number of times strontium is concentrated by *Carteria* and *Nitzschia* over the amount occurring in sea water was determined. It was demonstrated that the division rate, the age of the culture, and the hydrogen ion concentration all influenced the number of times strontium was concentrated in *Carteria* cells.

Exchange experiments showed that radioactive strontium was not lost from *Carteria* cells unless the medium contained a chelating compound. Some loss of radioactive strontium from *Carteria* cells occurred when the cells were filter-washed with several different types of solutions.

## INTRODUCTION

Strontium resembles calcium chemically and has been found in all phases of the biosphere in which analyses have been made (Odum 1951b). Although strontium moves in a biogeochemical cycle resembling that of calcium, it occurs in much smaller amounts in most phases. The concentration of strontium in sea water has been reported over a wide range of values and has been shown to be a conservative element varying with salinity. At 35‰ the preferred concentration for strontium is 8.10 mg/l (Odum 1951a).

The extensive distribution of strontium in the biosphere and the formation of radioactive strontium by nuclear detonations have resulted in a greater number of investigations into its role in the biotic cycle. Also the metabolism of strontium has been investigated because radioactive strontium is considered potentially one of the most hazardous of the fission products. There are several criteria by which a given fission product can be judged biotically important. Probably the three most important are its yield in the fission process, which controls the amount occurring as a pollutant in water, its half-life, and its biological absorption and retention by the organism.

With the possibility of natural bodies of water becoming polluted with radioactive strontium, it is important that the strontium cycle in aquatic environments be better understood. In the oceans the planktonic algae, because of their abundance, probably remove larger quantities of mineral elements from the water than any other form. Some elements are taken up in such large quantities that they are concentrated considerably over their amounts in sea water. Since the uptake of radioactive isotopes is usually similar to that of the nonactive isotopes of the element, it is possible that the contamination of marine waters with fission products would, in many instances, result in a rapid concentration of the activity in the planktonic algae. Consequently, animals feeding upon the algae could be expected soon to incorporate into their bodies a large percentage of the radioactivity. Thus the tracing of strontium through the biotic cycle in marine waters should begin with studies of its uptake from the water by planktonic algae.

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[1] The work reported was carried on as a part of a cooperative project of the U. S. Fish and Wildlife Service and the U. S. Atomic Energy Commission. Publication has been approved by these Agencies.

The uptake of strontium by plants and the factors controlling it have been investigated by a number of workers using radioactive strontium as a tracer. The accumulation of radioactive strontium by plants from three different types of soils has been found to be influenced by both the hydrogen ion concentration and the exchangeable calcium content of the soil (Romney *et al.* 1954). In addition to these factors, Rediske and Selders (1953), working with higher plants grown under controlled conditions of nutrient culture, observed that the strontium concentration also influenced the amount taken up by the plants. Spooner (1949) measured the concentration of strontium in several species of large marine algae. Also Knauss and Porter (1954) followed the uptake of radioactive strontium by the freshwater planktonic alga, *Chlorella*. It has been reported that in some species of plants strontium reacts much as calcium, and in *Chlorella* in particular, Walker (1953), using chemical methods of measuring strontium, observed that it could be substituted quantitatively for calcium. Even though strontium is taken up by most organisms, it is not considered an essential element for either plants or animals (Comar 1955).

Radioisotopes have been used in measuring the uptake and for elucidating the role of certain elements in the metabolism of various organisms. Investigations such as these have required the use of only one isotope at a time. However, situations may arise where it is necessary to follow the simultaneous uptake of two or more elements from the medium. Wherever pollution occurs, the possibility of more than one radioisotope occurring in the water will almost always be present. For this reason a mixture of the radioisotopes of strontium, Sr$^{89}$ and Sr$^{90}$, and yttrium, Y$^{90}$, was used in this investigation.

The purpose of this investigation was to measure the percentage of activity due to strontium and yttrium in cells grown in the presence of these radioactive isotopes, to determine those factors influencing the uptake of strontium, to find the concentration factor for strontium in *Carteria* when grown under different conditions, and to consider the importance of algae in passing radioactive strontium into higher trophic levels.

The author wishes to thank Dr. John H. Ryther for the *Nannochloris* and *Porphyridium* cultures, and Mr. Albert W. Collier for the *Gymnodinium* culture.

## MATERIALS AND METHODS

The following planktonic algae were used in this investigation:

CHLOROPHYCEAE
  *Chlamydomonas* sp.
  *Carteria* sp.
  *Thoracomonas* sp.
  *Chlorella* sp.
  *Nannochloris atomus*
CHRYSOPHYCEAE
  *Chromulina* sp.
BACILLARIEAE
  *Amphora* sp.
  *Nitzschia closterium*
  *Navicula* sp.
DINOPHYCEAE
  *Gymnodinium splendens*
  *Gyrodinium* sp.
RHODOPHYCEAE
  *Porphyridium cruentum*

Only the *Nitzschia closterium*, *Chlamydomonas*, *Carteria*, *Nannochloris* and *Porphyridium* were pure cultures. The other species were in a high state of purity, having been grown only in inorganic medium, and thus were probably sufficiently free from bacteria to make their use valid.

Radioactive strontium occurs as 12 different isotopes. The radioactive mixture which was supplied on authorization from the Isotopes Division, United States Atomic Energy Commission, and used in this investigation consisted of about 90 per cent Sr$^{89}$ and 10 per cent Sr$^{90}$. The values currently listed for the half-life of Sr$^{90}$ ranges from 19.9 years to 28.5 years, with 25 years being used most often as the average half-life. The Y$^{90}$ which is formed through the decay of Sr$^{90}$ has a half-life of 61 hours. After Sr$^{90}$ has decayed for a period of time, a steady state is reached, known as secular equilibrium, where the formation of Y$^{90}$ just equals its decay. The half-life of the mixture was 54 days when received at our laboratory.

Only an insignificant amount of any $Y^{90}$ taken up by the cells would remain after 10 half-lives, or after about 22 days. Also the $Sr^{89}$, $Sr^{90}$, and nonactive strontium enter the cells in the same proportions as that in the culture medium, since the cells can not distinguish between these isotopes. Thus, after the $Y^{90}$, which entered the algae as such, decays, a state of secular equilibrium is reached in the cells. In the following experiments the activity remaining in the cells after 22 days is referred to under the collective term of "radioactive strontium". While this activity will be due to $Sr^{89}$, $Sr^{90}$, and $Y^{90}$, it all entered the cells as strontium and can be interpreted as radioactive strontium present at the time involved.

Samples of cells which were separated from the medium by filtering on to millipore filter paper discs were used for radioactivity measurements. These discs were then placed in plastic Petri dishes. Unless otherwise stated, the radioactivity in the cells was measured with an end-window Geiger-Müller tube connected to a scaler. Appropriate corrections were made for geometry, coincidence, background, and decay. Radioactivity measurements on all samples were made for a sufficient length of time to give a one per cent error. The thickness of the different samples never varied enough to make any significant change in the activity determination for the cells.

Natural sea water enriched with Miquel's Solutions (Rice 1953) was used in all experiments where the calcium and strontium concentrations were not controlled. When it was necessary that only certain amounts of these two elements appear in the medium, the algae were grown in an artificial sea water. This sea water is similar to that of McClendon *et al.*, described in Sverdrup, Johnson, and Fleming (1942), and that used by Ryther (1954). It was prepared by the following formula with the amounts listed in parts per thousand:

| | | | |
|---|---|---|---|
| $NaCl$ | 26.5 | $KNO_3$ | 0.1 |
| $MgCl_2 \cdot 6H_2O$ | 2.5 | $SrCl_2 \cdot 6H_2O$ | |
| $MgSO_4 \cdot 7H_2O$ | 3.3 | $NaSiO_3 \cdot 9H_2O$ | 0.023 |
| $CaCl_2 \cdot 2H_2O$ | | $KH_2PO_4$ | 0.0075 |
| $KCl$ | 0.7 | $MnCl_2 \cdot 4H_2O$ | 0.0033 |
| $NaHCO_3$ | 0.2 | $ZnSO_4 \cdot 7H_2O$ | 0.00001 |

Algal cultures were grown in Erlenmeyer flasks of a capacity twice the volume of the medium used. After the appropriate number of cells and the desired amount of radioactivity had been added to the medium, the cultures were grown in a culture cabinet (Rice 1953) in constant illumination with daylight fluorescent lamps and at a temperature of 22 ± 2°C.

The number of cells per liter was determined after vigorously shaking the culture to distribute the cells evenly throughout the medium. The average of two cell counts with an improved Neubauer haemocytometer was taken as the population size. A careful check has shown that this method is very accurate for determining sizes of unialgal populations.

The water content of seven species of algae was found to be 90 per cent of the wet weight with a maximum variation of 2.2 per cent. This was obtained by first removing large quantities of cells from the medium by centrifuging for 2 to 3 hours. After the medium was decanted, the tubes were left inverted for several minutes so that water could drain from the surface of the cells packed in the bottom of them. The surface layer of cells was carefully removed and the remaining cells were placed on a very light weighing disc. Several pieces of highly absorbent paper were inserted into the mass of cells to remove any water which might have remained. They were then weighed and dried to a constant weight in a vacuum oven at 55°C. The wet weights of cells reported in these experiments were calculated on dry weight measurements.

## EXPERIMENTAL RESULTS

### *Uptake of radioactivity by various species*

Twelve species of algae were grown in the $Sr^{89}$, $Sr^{90}$, and $Y^{90}$ mixture to measure whether or not there is a selective uptake of strontium or yttrium. Any difference in the uptake of these two radioisotopes would upset the secular equilibrium. In this parent-daughter relationship the decay rate of the parent and daughter isotopes are very different making it possible to observe selective uptake by following the decay curves of the radioactivity in the algal cells.

The algae were prepared in separate cultures with as much as 10 microcuries of the radioactive mixture per liter of medium and were grown in the culture cabinet for 10 to 12 days. Enough cells to give a rather high radioactive measurement were then filtered and the radioactivity in them determined immediately. The decay of the radioactivity in the cells was followed by periodically measuring the activity remaining in the samples for a period of 30 days, and the results are shown in Figure 1.

It was found that 10 of the 12 species investigated selectively separated $Y^{90}$ from $Sr^{90}$ (Fig. 1). From the decay curve of the radioactivity in *Chlamydomonas*, one of these 10 species, it can be seen that the activity in the cells on the 22nd, 25th, and 30th days was the result of strontium uptake. Since these three points represent the decay of the radioactive mixture in secular equilibrium, a straight line can be drawn through them and extended to zero time where it intersects the ordinate at a point representing 6.5 per cent. This can be interpreted to mean that, of the total activity in the *Chlamydomonas* cells at the time of the first determination, 6.5 per cent was due to radioactive strontium and 93.5 per cent was the result of $Y^{90}$ uptake. The darkened curve, which includes the decay curves of all 10 species, indicates that most of the activity was due to $Y^{90}$ uptake. Table 1 shows the exact per cent of radioactive strontium and $Y^{90}$ in these 10 species of algae plus that occurring in *Carteria* and *Thoracomonas*, which were also grown and tested under similar conditions to those described above.

TABLE 1. *Differential uptake of radioactive strontium and yttrium by algae*

| Species | Percentage activity from strontium | Percentage activity from yttrium |
|---|---|---|
| *Carteria* sp. | 100.0 | |
| *Thoracomonas* sp. | 50.4 | 49.6 |
| *Amphora* sp. | 10.0 | 90.0 |
| *Navicula* sp. | 8.5 | 91.5 |
| *Chromulina* sp. | 8.2 | 91.8 |
| *Chlamydomonas* sp. | 6.5 | 93.5 |
| *Nitzschia closterium* | 6.0 | 94.0 |
| *Nannochloris atomus* | 5.7 | 94.3 |
| *Chlorella* sp. | 5.3 | 94.7 |
| *Porphyridium cruentum* | 4.4 | 95.6 |
| *Gymnodinium splendens* | 4.1 | 95.9 |
| *Gyrodinium* sp. | 2.3 | 97.7 |

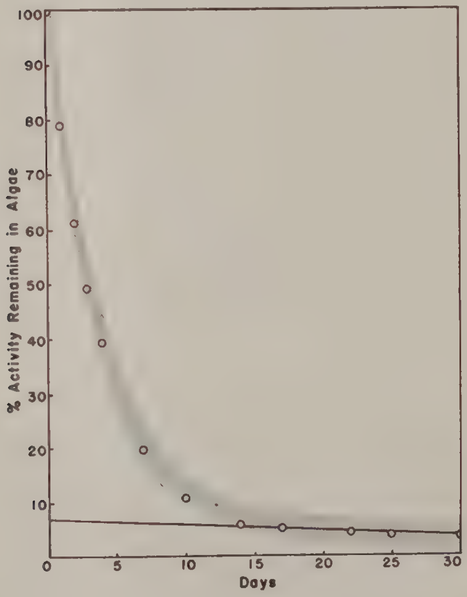

FIG. 1. The decay curves for radioactivity contained in ten of the twelve species tested fell within the darkened curve. The circles indicate the decay of radioactivity in *Chlamydomonas cells*.

With the exception of *Carteria* and *Thoracomonas*, the other species of algae concentrated radioactive yttrium to a much greater extent than radioactive strontium. The amount of $Y^{90}$ in the medium could not have represented much of the total activity, for only 10 per cent of the original mixture was $Sr^{90}$ from which the $Y^{90}$ originated. Since the $Y^{90}$ taken up by some species represented up to 97 per cent of all the activity in them, it can immediately be seen that only a very small per cent of the radioactive strontium was removed from the medium by these cells.

Since there is such a small amount of yttrium in sea water, its adsorption on the surface of the cells would be important in its removal from the medium. The *Carteria* cells did not appear to take up much yttrium, if any, yet these cells also present a large surface area, the same as the other cells, on which the yttrium could be adsorbed. The decay of radioactivity in the *Carteria* cells showed no significant deviation from the decay of the radioactive mixture, which would indicate that the radioisotopes in the *Carteria* cells after 10 to 12 days occurred in about the same proportions. If most of the $Y^{90}$, as well as the radioactive strontium, had been removed from the medium by the *Carteria* cells, then the decay of the activity in them could not vary significantly from that of the radioactive mixture.

### *Uptake of radioactivity by* Carteria

Cultures of *Carteria* were prepared and the radioactive mixture was added to the medium so that the uptake of $Y^{90}$ could be measured more carefully. These cultures were sampled periodically and the radioactivity of the separated cells was measured immediately and again after an interval of time equal to 10 half-lives of $Y^{90}$. From the difference in the radioactivity of the cells at the time of the initial determination and that after decay of the $Y^{90}$, the per cent of the radioactivity due to strontium and yttrium was calculated for the different times of exposure.

The uptake of these elements is shown in Table 2. The amount of radioactive strontium removed from the medium by the cells during the first 15 minutes is insignificant in relation to the amount in the cells after 72 hours. The uptake of $Y^{90}$ was quite different, since more than half the $Y^{90}$ in the cells after 72 hours was present after only 15 minutes. Even though the $Y^{90}$ in the cells increases for each interval of time, it may not be significant, since the $Y^{90}$ counts later in the experiment were obtained by getting the difference between two large numbers. When the $Y^{90}$ counts in the cells are converted to represent the per cent of the total counts in them, it can be seen that, after 15 minutes, about 90 per cent is due to $Y^{90}$, while after 72 hours the $Y^{90}$ accounts for only about 3 per cent.

TABLE 2. *Uptake of radioactive strontium and yttrium by* Carteria *cells*

| Hours | Strontium activity (counts/min) | Yttrium activity (counts/min) | Percentage activity from yttrium |
|---|---|---|---|
| ¼ | 69 | 592 | 89.56 |
| 6 | 527 | 735 | 58.24 |
| 24 | 4,800 | 848 | 15.01 |
| 48 | 23,619 | 898 | 2.47 |
| 72 | 34,769 | 948 | 2.65 |

### *Uptake of strontium by* Carteria *as related to cell condition*

*Dead cells.* Cells were first killed by either heating the medium to 95°C and holding it at this temperature for 5 minutes or by adding glacial acetic acid, 1 cc per 100 cc of culture medium. After 30 minutes the cells were centrifuged from the medium containing the glacial acetic acid and resuspended in new culture medium. It was now possible to follow the uptake of radioactive strontium by cells killed in two different ways and to detect any change in the permeability of the cell membrane. The remainder of the cells, which were untreated, were retained as a control. To each of the three types of cultures 10 microcuries of the radioactive mixture were added per liter. The activity in the cells, which were sampled periodically, was not measured until after an interval of time equal to 10 half-lives of $Y^{90}$.

The cells in the control continued to take up radioactive strontium, while the strontium content of neither the acid treated nor the heated cells increased significantly after 15 minutes (Table 3). There are fewer counts for the acid treated than for the heated cells, since there was an unavoidable loss in centrifuging cells from the acid medium and resuspending them in the standard medium. It can be concluded from this experiment that the uptake of radioactive strontium by cells killed by these methods occurs instantaneously and is due to adsorption or exchange since it does not continue with time. Also the activity taken up by living cells during the first 15 minutes must

TABLE 3. *Comparison of radioactive strontium accumulated by living and dead cells*

| Hours | Radioactivity in cells (counts/min) | | |
|---|---|---|---|
| | Living | Acid killed | Heat killed |
| ¼ | 64 | 38 | 70 |
| 24 | 5,658 | 37 | 56 |
| 48 | 11,814 | 36 | 69 |

TABLE 4. *Comparison of uptake of radioactive strontium by non-dividing and dividing cells*

| Hours | Dividing | | Non-dividing | |
|---|---|---|---|---|
| | Population (cells × $10^7$) | Radio-activity (counts/min) | Population (cells × $10^7$) | Radio-activity (counts/min) |
| ¼ | 6.3 | 67 | 6.3 | 54 |
| 6 | | 846 | | 66 |
| 24 | 20 | 6,226 | 6.3 | 180 |
| 48 | 52 | 34,744 | 6.3 | 118 |
| 72 | 91 | 75,830 | 6.3 | 82 |

be due to adsorption or exchange, since it was not significantly larger than that removed by the dead cells.

*Dividing cells.* The amount of strontium removed from the medium due to growth can be measured by comparing the amount of radioactive strontium taken up by a dividing cell with that of a non-dividing cell. A culture of *Carteria* cells was placed in the dark for 7 days during which time cell division ceased. Two new cultures were started from this culture with 9 microcuries of the radioactive mixture per liter. One was retained in the dark and the other was placed in the culture cabinet in the light. The cell population size increased rapidly in the culture placed in the light, while there was no detectable change in the population size in the culture kept in dark (Table 4). The radioactive strontium continued to enter the cells in the light, while there was no further increase in uptake after 24 hours by the cells kept in the dark. The amount of radioactive strontium taken up as a result of adsorption and/or exchange by each cell in the light, if related to time, could not be greater than that taken up by a cell in the dark. The average amount of radioactive strontium taken up *per cell* in the light was 64 times as much as that taken up by a cell in the dark. It was shown in the control of the previous experiment that the amount of radioactive strontium taken up by cells increases as the population size increases. It has now been demonstrated in this experiment that dividing cells remove much more radioactive strontium *per cell* from the medium than non-dividing cells. Thus the greatest uptake of strontium must be due to or related with absorption and the formation of new protoplasm.

TABLE 5. *Effect of division rate and age of culture on accumulation of radioactive strontium*

| Day | Rate (divisions/day) | | Strontium (counts/min/$10^4$ cells) | |
|---|---|---|---|---|
| | Culture 1 | Culture 2 | Culture 1 | Culture 2 |
| 1 | 1.59 | 1.00 | 6.2 | 3.4 |
| 2 | 1.30 | 0.30 | 13.8 | 8.2 |
| 3 | 0.75 | 0.57 | 16.6 | 9.0 |

*Cells dividing at different rates.* The question now is whether there is any difference in the amount of radioactive strontium *per cell* when the cells are dividing at different rates and when the cultures have grown for different periods of time. Culture 1 was started with 6.3 × $10^7$ cells per liter from a culture which had been kept in the dark prior to this experiment. Culture 2, prepared with cells grown in the light in medium containing no phosphorus, was started with 19 × $10^7$ cells per liter. Both cultures were started with 9 microcuries of the radioactive mixture per liter.

Cells in Culture 1 had a faster rate of division than Culture 2 (Table 5). Also the amount of radioactive strontium in each cell in Culture 1 at the end of each interval of time was larger than that occurring per cell in Culture 2. From the data presented in Table 5 it appears that the faster the rate of division, the faster radioactive strontium enters a cell. It is of further interest to note that, regardless of the rate of division, more radioactive strontium occurs in a cell the older the culture at the time of sampling. At least this holds for the intervals of time tested in this experiment.

### *Uptake of calcium and strontium by* Carteria *in relation to their concentrations in the medium*

To find the effect of the calcium concentration on the uptake of strontium and vice versa, artificial sea water was devised in which the calcium and strontium concentrations could be varied. The growth of *Carteria*, as well as that of several other species, was as good as, or better than, that obtained in culture medium prepared by adding nutrients to natural sea water.

*Effect of calcium concentration on uptake of* *strontium.* Whether or not the calcium concentration has any effect on the uptake of strontium was followed in *Carteria* cultures which were prepared with the same size inoculation of cells but with different concentrations of added calcium (Table 6). The strontium concentration was the same in all cultures, and 5 microcuries of the radioactive mixture were added per liter. Thus the uptake of a given per cent of radioactive strontium in any culture represented the removal of the same total amount of strontium. In Table 6 it can be seen that about the same per cent of radioactive strontium was removed from the culture medium with the three highest concentrations of calcium. The cells in the cultures with 0.075 grams of calcium per liter removed a little more strontium, while the cells in cultures with no added calcium took up the largest per cent of strontium. It should be pointed out that the number of cells obtained in all cultures with added calcium did not vary significantly from $56 \times 10^7$ cells per liter, while cultures with no added calcium reached a population of only $8 \times 10^7$ cells per liter. Since without added calcium in the medium the cells could not divide even in the presence of excess strontium, it can be concluded that strontium cannot be substituted for calcium.

In the cultures with no added calcium there were fewer cells and somewhat more uptake of strontium; thus the amount of strontium *per cell* was considerably more. Since calcium is closely related with the permeability of the cell membrane, the added uptake of strontium in the cultures with the lower calcium concentrations may be the result of a change in permeability of the membrane.

*Effect of strontium concentration on uptake of calcium.* The effect of strontium on the uptake of calcium by *Carteria* cells was tested by starting cultures with the same size inoculation of cells in artificial sea water containing different concentrations of added strontium (Table 7). The calcium concentration was the same in all cultures, and 9 microcuries of $Ca^{45}$ were added per liter. In Table 7 it is shown that about the same per cent of $Ca^{45}$, and therefore total calcium, was removed from all cultures, regardless of the strontium concentration. It can be concluded that the uptake of calcium by *Carteria* cells is not influenced by these concentrations of strontium.

TABLE 6. *Effect of calcium concentration on uptake of strontium*

| Calcium added (g/l) | Percentage radioactive strontium in cells |
|---|---|
| 0.300 | 40.0 |
| 0.275 | 38.6 |
| 0.150 | 38.9 |
| 0.075 | 45.6 |
| 0.000 | 54.1 |

TABLE 7. *Effect of strontium concentration on uptake of calcium*

| Strontium added (g/l) | Percentage radioactive calcium in cells |
|---|---|
| 0.011 | 1.5 |
| 0.008 | 1.3 |
| 0.005 | 1.4 |
| 0.003 | 1.1 |
| 0.000 | 1.3 |

The population size reached in the cultures to which no strontium was added was $50 \times 10^7$ cells per liter, while in all cultures to which strontium was added it did not vary significantly from $60 \times 10^7$ cells per liter. It was shown in the previous experiment that cells could not divide without added calcium Here it can be seen that cells divide almost as fast in the absence of as in the presence of *added* strontium. It appears that strontium is not very necessary for the growth of *Carteria*, or if needed, then small amounts of strontium entering the artificial sea water as impurities are sufficient to meet the strontium requirements, or, finally, that calcium can be substituted for strontium.

*Effect of strontium concentration on uptake of strontium.* *Carteria* cells were grown in three different concentrations of strontium in order to determine the effect of the concentration in the medium on the uptake by the cells. Three artificial sea water media were prepared: one with 11 milligrams of strontium and 5.4 microcuries of the radioactive mixture per liter, one with 55 milligrams of strontium and 27 microcuries per liter, and one with 110 milligrams of strontium and 54 microcuries per liter. Cultures for each concentration were inoculated with

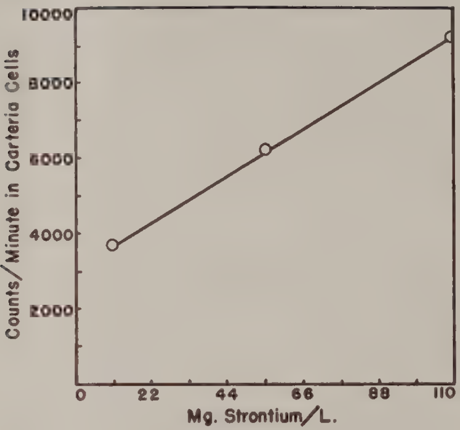

FIG. 2. Uptake of strontium in relation to its concentration in the medium.

the same number of cells. After eight days in the culture cabinet in the light, the cells were filtered from the medium and the radioactivity in them was measured. The specific activity was the same in each culture; therefore, the uptake by the cells of a given amount of activity in any culture represented the uptake of the same amount of total strontium.

The uptake of strontium by the cells increased as the concentration of strontium in the medium increased, and the amounts of strontium taken up at these three concentrations formed a straight line when graphed (Fig. 2). A five fold increase of strontium in the medium caused, roughly, a doubling of the amount of strontium in the cells, while 10 times as much strontium in the medium resulted in a tripling of the quantity of strontium in the cells. The number of cells per liter at all three concentrations did not vary significantly from each other. It can also be concluded from these observations that concentrations of strontium as high as these exert no toxic effects upon the division rate of *Carteria.*

### *Conditions affecting the concentration of strontium in* Carteria *cells*

The concentration of an element by a marine organism can be determined more easily, in most instances, with radioisotopes than by ordinary chemical analysis. The radioisotope of the element to be studied is first added to a known volume of sea water. The radioactivity per aliquot volume of water is then measured and the test organism is placed in the water. The organism must remain in the active sea water until no more activity enters it and until a state of equilibrium is reached between the activity in the water and that in the organism. At this time the isotope will have been distributed at random wherever the element occurs. After a state of equilibrium has been reached, as determined by measuring the activity in aliquot samples of water removed periodically, the activity remaining in the water is compared with that originally present. The volume of sea water which would have been necessary to add to the original volume to reduce the activity in an aliquot portion to the same level as that resulting from the addition of the organism is now computed. The wet weight of the organism is then divided into the weight of this volume of water to determine the number of times the organism has concentrated the element. Since there is a radioactive parent element producing a radioactive daughter, the extent to which one element is concentrated is more difficult to determine than when only one radioisotope is used, but can be ascertained by following the decay curve. The method used was developed by Spooner (1949) for measuring the concentration of strontium by large algae.

*Rate of division.* In a previous experiment it was found that more strontium was taken up *per cell* when the rate of division was faster. It was now of interest to determine whether faster dividing cells will also concentrate more strontium per unit weight. Two identical cultures of *Carteria* were prepared with 12.3 microcuries of the radioactive mixture in each culture. One culture was placed in the culture cabinet in the light and the other was grown in natural daylight. The cells grown in natural daylight divided at a much slower rate with 5.18 grams of cells being harvested after 24 days, while there were 5.20 grams of cells after 13 days in the culture grown in the culture cabinet. After these intervals of time samples of medium from which the

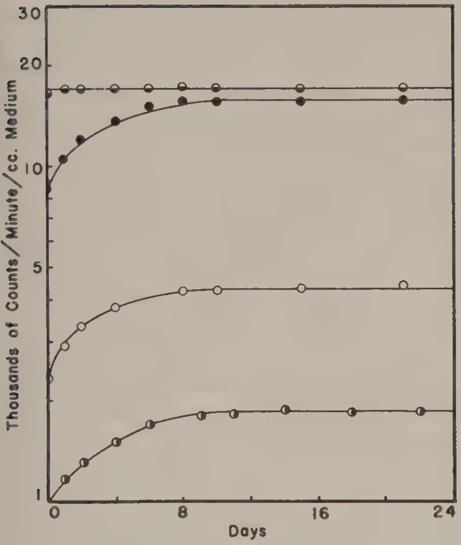

FIG. 3. Decay curves for medium in which *Carteria* and *Nitzschia* were grown.

- ◒ Control medium in which no cells were grown
- ● Medium in which *Nitzschia* was grown in culture cabinet
- ○ Medium in which *Carteria* was grown in natural daylight
- ◑ Medium in which *Carteria* was grown in the culture cabinet

cells had been removed by filtering were dried and measured for their radioactivity. A control had been prepared at the beginning of the experiment by adding 12.3 microcuries of the radioactive mixture to a liter of culture medium containing no *Carteria*. Radioactivity in samples of medium from the *Carteria* cultures were counted periodically and compared with samples prepared in a similar manner from the control and counted over the same interval of time. Cultures of *Nitzschia* had also been prepared with 12.3 microcuries of the radioactive mixture per liter and grown in the culture cabinet and in natural daylight. Preparation and counting of radioactivity in the medium was similar to that used for the control and for *Carteria*. The decay curves for the control, for the *Carteria* cultures grown in the cabinet and in the window, and for the *Nitzschia* grown in the culture cabinet are shown in Figure 3. The radioactive mixture had been in our laboratory for some time prior to this experiment, and most of the $Sr^{89}$ had decayed, as revealed by the insignificant drop in the number of counts per minute in the control samples over the interval of 22 days during which counts were made. This longer half-life indicated most of the radioactive strontium present was $Sr^{90}$.

The decay curves for the medium which contained the algae show an increase in radioactivity for about 8 to 10 days. This was due to more $Y^{90}$ being formed by the decay of $Sr^{90}$ than was decaying. After secular equilibrium was reached in the *Carteria* and *Nitzschia* culture media, the decay curves level off. From this time on the rate of decay is similar to that of the control. The per cent of the original activity remaining in the *Carteria* and *Nitzschia* culture media at this time was calculated and the concentration factors computed. The formula for determining the concentration factor is as follows:

$$\frac{\text{Weight of medium} \times \text{Per cent activity in cells}}{\text{Wet weight of cells} \times \text{Per cent activity in medium}} = \text{Concentration factor}$$

It was found that *Carteria* cells concentrated strontium to a greater extent when grown in the culture cabinet where cell division was faster. The concentration factor for cells grown in the cabinet was 1,616 while it was only 561 for those grown in natural daylight. Also, regardless of whether *Carteria* cells were grown in the cabinet or in natural daylight, they concentrated much more strontium than did *Nitzschia* cells. *Nitzschia* cells concentrated strontium only 17 times over the concentration occurring in sea water when grown either in the culture cabinet or in natural daylight.

*Age of culture.* The concentration factor for *Carteria* cells was followed in the same cultures over a period of several days. Since adsorption and exchange occur rather rapidly and the *Carteria* cells divide about once a day for the first few days, it was believed that within three days the radioactive strontium would be distributed

TABLE 8. *Increase of strontium concentration in* Carteria *with age of culture*

| Age in days | Concentration factor |
|---|---|
| 3 | 100 |
| 6 | 243 |
| 10 | 337 |
| 18 | 1,244 |

at random wherever strontium occurred. Thus the first determinations were made on the third day after the culture was started and again on the 6th, 10th, and 18th days. The methods used in this experiment are the same as those discussed in the previous one. The cells continued to increase in weight with time. Also, the per cent of radioactive strontium in the cells and the concentration factor for strontium continued to increase with time (Table 8).

*Hydrogen ion concentration.* The biological availability to algae of many elements for uptake and utilization in metabolic processes depends upon the element existing in an ionic state in the water. Since the ionic state of many elements is closely related to the hydrogen ion concentration, the availability of strontium to *Carteria* grown in culture medium with the hydrogen ion concentration initially adjusted to pH 6.0 and pH 9.0 was determined. The control consisted of a culture started with the regular culture medium which had a pH of 8.2. Hydrochloric acid was used to reduce the medium to pH 6.0 and sodium hydroxide was added to raise the medium to pH 9.0. The cultures contained 12.3 microcuries of the radioactive mixture.

*Carteria* cells grown in culture medium with pH 8.2 concentrated strontium 975 times over its amount in sea water. The concentration factor for cells grown in medium with an initial pH of 6.0 was only 223, while that in medium with a pH 9.0 was even less, being only 85. The cells in the culture with a pH 6.0 were larger and weighed more than those in the control culture, even though the cells in the control culture divided a greater number of times. All cultures were started with the same number of cells, but counts just prior to weighing showed the largest population in the control culture. Again the highest concentration factor occurs where there has been the greatest number of cell divisions. The lower concentration factors for cells grown at pH 6.0 and pH 9.0 may be due not only to the strontium being less available for uptake at these hydrogen ion concentrations, but may also be partially due to the reduced rate of division.

### *Loss of radioactive strontium from* Carteria *cells*

Whether an element remains in an exchangeable state once inside a cell depends upon the kind of compounds into which it is bound. If an element is loosely bound or bound into compounds with a rapid rate of turnover, then the possibility of exchange is greater. Also elements which remain in the cell in what has been referred to as the "metabolic pool" may be exchanged more easily than other elements bound in tissue.

*Carteria* cells which had grown in the presence of the radioactive mixture for 12 days were fractionated to determine the distribution of strontium. It was found that more than 97 per cent of the activity contained in the cells was soluble in TCA (trichloracetic acid). The fractionating procedure was similar to that used by Rice (1953). Also *Carteria* cells were fractionated in alcohol and in ether. Less than 0.1 per cent of the activity was lost from the cells to either solution.

*Resuspended in non-radioactive culture medium.* The loss of strontium from *Carteria* cells was followed by centrifuging cells from a 5-day-old culture which had been started in artificial sea water with 10 microcuries of the radioactive mixture per liter, and resuspending them in another portion of artificial sea water containing no radioactive strontium. This culture was then divided into two cultures and to one enough strontium was added to give the same concentration as occurs in natural sea water. The cultures were returned to the culture cabinet and the medium was sampled after 1, 5, 24, and 48 hours. The radioactivity was measured in both the cells and medium under comparable conditions. The cells from 25 cc of medium at zero time contained 6651 counts per minute. From these data it can be computed that less than 2 per cent of the radioactivity in the cells was lost to the medium, even after 48 hours.

*Resuspended in culture medium containing a chelating substance.* The radioactive strontium contained in the cells that were resuspended in nonactive medium in the previous experiment may have moved back and forth between the cells and medium, even though it could not be detected in the medium. Thus the experiment was repeated with a chelating compound, Versene, a sodium salt of ethylene-diamine-tetraacetic acid, added to the medium. This compound can bind elements leaving cells into such large molecules that they can not reenter the algae. The use of chelating compounds has been extensively studied by Hutner, Provasoli, Schatz, and Haskins (1950). With chelating agents metal ions can be reduced in cultures to levels that are not harmful, or deficiencies of some elements may be created so complete as to reduce the division rate.

A *Carteria* culture was prepared in artificial sea water with 10 microcuries of the radioactive mixture per liter. After growing in this medium for 7 days, the cells were centrifuged and resuspended in another volume of artificial sea water containing neither active nor nonactive strontium. This culture was divided into two parts and different amounts of the chelating agent were added to each (Table 9). The cells were filtered out of the medium, and the radioactivity in the medium was counted with a dip-type Geiger-Müller counting tube at frequent intervals of time over a 24-hour period (Table 9). It was found that radioactive strontium did migrate out of the cells, the amount in the medium increasing with time for the first two hours. A greater amount of radioactive strontium, and therefore total strontium, was found in the medium containing the larger quantity of Versene.

TABLE 9. *Loss of radioactive strontium from* Carteria *cells resuspended in nonactive medium containing a chelating substance*

Cells from sample volume initially contained 8391 counts per minute.

| Hours | Radioactivity in filtered medium (counts/min ) | |
|---|---|---|
| | With 0.5 g Versene/l | With 1.0 g Versene/l |
| ½ | 764 | 1694 |
| 1 | 1223 | 2646 |
| 2 | 1654 | 3226 |
| 4 | 1550 | 3408 |
| 7 | 1675 | 3425 |
| 24 | 1525 | 3250 |

TABLE 10. *Loss of radioactive strontium from living and dead* Carteria *cells when filter washed with different types of solutions*

| Washing solution | Percentage loss | |
|---|---|---|
| | Living cells | Dead cells |
| Culture medium | | 15.49 |
| Hot culture medium | | 24.77 |
| Culture medium (with 10X Sr in sea water) | 2.09 | |
| Culture medium (pH 5.4) | 7.73 | |
| Culture medium (pH 3.0) | 29.52 | |
| Culture medium (pH 2.0) | 37.85 | |
| Distilled water | 7.19 | 13.65 |
| Hot distilled water | 24.20 | 24.10 |

*In filter-washing.* The instantaneous loss of strontium from both living and dead cells can be conveniently measured by a filter-washing technique. In these experiments the *Carteria* cells, after growing 8 to 12 days in medium containing up to 10 microcuries of the radioactive mixture per liter, were filtered on millipore filter discs and washed with different types of medium. The radioactive strontium remaining in the cells after washing was compared with that present before washing to determine the per cent lost. Cells, first killed by drying at 70°C for 4 hours after having been suspended on the filter, were also washed to determine whether more strontium would be exchanged or lost from dead cells such as these or from living cells, since the permeability of the cell membrane may be different in dead cells.

There was not much difference in the amount of radioactive strontium lost from living and dead cells washed with distilled water (Table 10). In another experiment it was found that only 35.4 per cent of the radioactive strontium was lost from living *Carteria* cells suspended in distilled water for as long as 65 hours. The per cent of radioactive strontium lost from living *Carteria* cells was large when filter-washed with culture medium having higher hydrogen ion concentrations than those occurring in sea water. It can be stated that most of the radioactive strontium contained in living cells is retained inside the cell, even when subjected to unfavorable media in filter-washing. Also, cells that have been dead for only a short period of time do not lose large quantities of radioactive strontium when filter-washed.

## DISCUSSION

In this investigation the algal cells were grown in medium containing $Sr^{89}$, $Sr^{90}$, and $Y^{90}$. Ten of the twelve species tested became more radioactive from the uptake of $Y^{90}$, which was present in very small amounts. In the other two, the quantity of $Sr^{89}$ taken up masked the uptake of the yttrium. This differential uptake was easily followed by observing the decay characteristics of the radioactivity of the cells. These observations emphasize that care must be taken in considering the radioactivity of biological materials when more than one radioactive element is used, even though one or more of the radioisotopes are present in trace amounts.

It should also be mentioned that upon addition of the radioactive mixture to sea water some yttrium occurs as a radiocolloid. Some of this colloidal material can be filtered and centrifuged from the culture medium. However, when the radioactive material is added to cultures of algae the amount obtained by either filtering or centrifuging the cells from the medium is several times that obtained in the absence of the cells. Since there is such a small amount of yttrium in sea water and since its removal by the cells is instantaneous, it is probably correct to assume that almost all of the yttrium is taken up by the algae.

The surface area of algal cells plays a very important role in removal of some nutrients from culture medium. In most investigations the uptake of an element is usually reported without much consideration of the amount adsorbed on the surface. For many elements adsorption on the surface may be only the precursor to its entry into the cell, but for other elements that cannot penetrate the cell membrane, or do so slowly, it may be a "retention area". The adsorption of an element onto the surface of a cell may be most important for those elements occurring in the medium in relatively small amounts or for those elements taken up by the cell in small quantities. If only a small trace of the element occurs in the medium, then the large surface area available on the cells may result in most of the element being adsorbed on the cell, regardless of whether or not it serves as a nutrient. On the other hand, if not much of an element is utilized in metabolism, the adsorption of small amounts on the surface may represent a significant portion of that taken up by the cell. In this last instance the actual requirement of the cell for the nutrient would be considerably less than the amount removed from the medium.

It was found that the adsorption of yttrium was significant in its total uptake, while the surface area retained an insignificant portion of strontium when compared to that entering the cells. The uptake of yttrium was almost complete within 15 minutes after adding the radioactivity. As far as could be ascertained no data have been published showing the need for yttrium in the metabolism of algae. While some of the $Y^{90}$ uptake may have been due to exchange, Spooner (1949) believes uptake as a result of adsorption in higher marine algae greatly exceeds that taken up through exchange. There are numerous other elements occurring in sea water in trace amounts which could also be adsorbed in considerable amounts just as readily as yttrium. The importance of adsorption on the surface of cells should be investigated further, since this may be the only explanation in some instances for the occurrence of some trace elements in algae. Stiles (1951) has stated that analysis has shown higher plants to contain small amounts of many elements not generally regarded as essential.

Very little strontium was taken up during the first 15 minutes in these experiments. Living cells, during the first 15 minutes, were found to take up no more activity than dead cells. Also living but non-dividing cells after 72 hours contained only one sixty-fourth the amount of strontium *per cell* as in dividing cells. If all the uptake by the non-dividing cells was due to adsorption, it can be seen that it still would be a very small per cent of that taken up by dividing cells. It seems that surface adsorption of strontium is of very minor importance in considering the uptake of this element.

The possibility of the *division rate* influencing the amount of an element in a cell has been considered in this investigation. It was found that during the first 72 hours more strontium entered a cell the faster the rate of division. Also it was shown that the concentration factor for strontium was larger for cells grown in the culture cabinet where cell division was more rapid than for cells grown in natural daylight. Krauss (1953), in measuring the nitrogen accumulation by *Scenedesmus* in medium with and without the three micronutrients, boron, zinc, and manganese, found that the uptake of nitrogen proceeded almost as rapidly in the cells where growth was limited by micronutrient deficiency as in cells growing at a normal rate. This resulted in the uptake of more nitrogen per cell in the culture medium with a nicronutrient deficiency or in the culture with the slower rate of division. That the rate of division may have no effect on the amount of an element *per cell* was observed in the uptake of cesium by *Nannochloris* cells (Rice, unpublished). In this experiment the uptake curve for cesium was parallel to the growth curve, indicating the uptake of an equal amount of cesium with the formation of a given quantity of cells throughout the entire growth phase.

From the data presented above it can be concluded that some elements enter cells in larger amounts when the division rate is faster, while other elements occur in larger amounts in more slowly dividing cells. Finally, there are some elements that enter cells at the same rate regardless of the division rate. It is usually difficult to change the rate of division without altering the chemical composition of the medium, so that the different rates of uptake mentioned as related to division rate could possibly be due to other factors. It is believed that this problem should be investigated further, and it is suggested that it could be accomplished either by starting cultures identically, except for the initial inoculation of cells, or by changing the intensity of light. It has already been shown many times that both the rate of division and total population size reached in algal cultures can be influenced by the number of cells used to start new cultures.

Walker (1953) showed that the calcium requirement in *Chlorella pyrenidosa* is almost quantitatively replaced by strontium. In a later investigation Walker (1954) found that closely related members of the Chlorophyceae differ widely in their ability to replace calcium with strontium. The experiments conducted in this investigation revealed that strontium could not be substituted for calcium in *Carteria*. It was further observed that calcium is essential for cell division and must be present in the medium before any significant amount of division occurs.

The calcium requirement for a marine diatom has been established at approximately 0.5 mg/100 cc culture medium (Hutner 1948). In the present investigation the effect of the strontium concentration upon calcium uptake by *Carteria* was measured with radioactive calcium. It was found that approximately the same per cent of total calcium was removed from the medium by *Carteria* cells. Since the artificial sea water was prepared with 0.3 grams of calcium per liter, the cells removed from 3.3 to 4.5 mg of calcium per liter. The highest of these amounts removed occurred in artificial sea water containing approximately the same concentration of strontium as sea water.

One of the most important factors affecting the amount of an element accumulated by planktonic algae is the concentration of the element in the culture medium. Ketchum (1939) has shown that the amount of phosphorus entering *Nitzschia closterium*, a marine diatom, depends upon the concentration of phosphorus in the medium, other factors being kept constant. The uptake of cesium by *Nannochloris*, a planktonic alga, was found to be proportional to the cesium concentration in the medium (Rice, unpublished). Also the accumulation of strontium in both higher plants (Rediske and Selders 1953) and in *Chlorella pyrenoidosa* (Knauss and Porter 1954) was found to be proportional to the concentration in the nutrient medium. The amount of strontium was also found to increase in *Carteria* cells as the concentration in the medium was increased. However, with a five-fold increase of strontium in the medium there was roughly a doubling of the amount of strontium entering the cells, and with ten times as much strontium in the medium there was approximately three times as much strontium taken up by the cells. The concentration of strontium tested with *Chlorella* was only over a range from 1.0 to 40 milligrams per liter, while in the present investigation the highest concentration used was 110 milligrams per liter. The smaller increase of strontium in *Carteria* cells than that observed in *Chlorella*, with an equal increase of strontium in the medium, was probably due to one or both of the following conditions: (1) the uptake of strontium by *Carteria* cells was less because two of the three concentrations tested were higher than those used by Knauss and Porter, and/or (2) the difference in the total quantity of nutrients and in their ratio one to another in the artificial sea water was such that less strontium was taken into the *Carteria* cells.

The amount of strontium found in plants appears to be related to the age of the plant. Rediske and Selders (1953) found the greatest concentration of strontium always in the oldest leaves, with a progressively smaller concentration as the age of the leaf decreased. While the age of leaves of higher plants may have no physiological property analogous to the age of a microorganism culture, in the present investigation it was observed that, regardless of the division rate, the amount of strontium per cell increased with the age of the culture for the time intervals tested. Also the concentration factor, which was determined after 3, 6, 10, and 18 days in one set of experiments, showed that more strontium occurred in a gram of cells after each interval of time. From these observations it appears that strontium continues to enter the cells with time, and that the amount entering the cells is not entirely related to the formation of new tissue. A possible explanation for this continuous uptake may be that the cells cannot prevent the strontium from entering and are not able to eliminate it, so that the amount per cell or per gram continues to increase with time.

The hydrogen ion concentration was found to control not only the rate of division of *Carteria* cells but also the amount of strontium concentrated by the cells. Whether the concentration of strontium was biologically unavailable due to the formation of certain strontium compounds or less strontium was removed because of the slower division rate of *Carteria* in the cultures with pH 6.0 and 9.0 is not known. The population in the culture started at a pH of 6.0 weighed more but had fewer cells than that in the culture started at pH 8.2.

It was found that no significant amount of radioactive strontium accumulated in the culture medium in which cells containing radioactive strontium were suspended. The results were the same whether or not the medium contained strontium. With the addition of a chelating agent the loss of radioactive strontium from the cells could be demonstrated. The order of chelation at a pH 8.2 is calcium, magnesium, and strontium. Also, it can be computed theoretically that it would require about 31.5 grams of Versene per liter to chelate any strontium leaving the *Carteria* cells. Since only two concentrations of Versene, 0.5 and 1.0 gram per liter, were used in the experiment reported here, it may seem improbable that chelation was responsible for the results observed. Nevertheless, radioactive strontium did leave the cells and accumulate in medium containing Versene. If not due to the phenomena of chelation it was brought about by some other change associated with the presence of the Versene. In this experiment the medium contained no strontium, so that this is not a demonstration of strontium exchange. Rather, it was only a short-circuiting of the strontium cycle between medium and cells, showing that strontium can leave the cells as well as enter them.

It was further shown that strontium is lost from *Carteria* cells by filter-washing. In this type of experiment media or other solutions used to wash the cells remain in contact with them for only a short interval of time. Appreciable quantities of radioactive strontium were lost from living cells to only three washing solutions: hot distilled water, culture medium with a pH of 2, and one with a pH of 3. Thus it appears that even though strontium may not be very important in the metabolism of the cells, it is not readily lost from them.

Some species of planktonic algae may not be very instrumental in concentrating and passing strontium into higher trophic levels. Of the twelve species tested, *Carteria* and *Thoracomonas* were the only forms that concentrated strontium to any great extent. The yield of cells in grams per liter is no doubt higher in laboratory cultures than ordinarily found in nature. A filter-feeding animal in sea water with a population of *Nitzschia closterium* as large as those obtained in these experiments could still obtain just over 3 grams of cells from a liter of water. Since the *Nitzschia* cells concentrated strontium only 17 times over its concentration in sea water, an animal filtering all the cells from a liter of water and incorporating all the strontium from the cells into its own body would only have a total amount of strontium equivalent to that occurring in about 51 cc of sea water. However, animals feeding upon *Carteria* cells would have a much larger supply of strontium from an equivalent weight of cells since *Carteria* was found to concentrate strontium up to 1616 times over the concentration in sea water. Other factors should be taken into consideration in discussing the role of phytoplankton as a source of strontium for animals, including the relatively high concentration of strontium in sea water and the large volume of water filtered by many filter-feeding animals. If these organisms can remove strontium from the sea water to meet any metabolic requirements they may have, it would appear that the added strontium from the phytoplankton food may not be a significant contribution.

## SUMMARY

1. All twelve species of planktonic algae grown in medium containing $Sr^{89}$, $Sr^{90}$ became radioactive. Only in *Carteria* and *Thoracomonas* was this radioactivity due chiefly to the uptake of radioactive strontium. The greatest part of the radioactivity in the cells of the other species was due to $Y^{90}$, the radioactive daughter of the $Sr^{90}$.

2. In *Carteria* there was almost instantaneous uptake of $Y^{90}$, which did not increase greatly with time and was therefore apparently the result of adsorption of $Y^{90}$ on the cell surfaces. The radioactivity resulting from the uptake of radioactive strontium was of little significance at first, but gradually it became the chief source of the radioactivity.

3. Dead and living, but non-dividing, cells did not remove any significant quantities of strontium from the medium. With dividing cells there was continued uptake; the faster the rate of division, the greater the amount of strontium taken up per cell during the first 72 hours after starting the culture.

4. Calcium at higher concentrations did not influence the amount of strontium taken up by *Carteria* cells. Also calcium was found to be essential for cell division. It was further found that strontium could not be substituted for calcium.

5. The strontium concentration did not influence the amount of calcium taken up by *Carteria* cells. When other factors were kept constant the amount of strontium removed by the cells was dependent upon the strontium concentration in the medium.

6. It was found that *Nitzschia closterium* concentrated strontium 17 times over its concentration in sea water. The concentration factor for strontium in *Carteria* was found to vary, being influenced by the rate of cell division, the age of the culture, and the initial pH of the medium in which the

cells were grown. However, it was much greater than that for *Nitzschia*.

7. Little radioactive strontium was lost or exchanged from *Carteria* cells when resuspended in non-radioactive medium. The addition of a chelating substance to the medium demonstrated that strontium does leave the cells. Also, very little radioactive strontium was lost from radioactive *Carteria* cells suspended on a filter and washed with various washing solutions.

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# CONSIDERATION OF MIDOCEAN FISH PRODUCTION AS RELATED TO OCEANIC CIRCULATORY SYSTEMS

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The sea receives most of the radiant energy reaching the earth simply because there is more sea than land, especially in middle and low latitudes where the angle of incidence is greatest and the loss by reflection is least. Therefore, so far as total absorption of energy is concerned, the sea's productive capacity should greatly exceed that of the land. Indeed Riley (1944) has estimated carbon production in the sea to be about 86% of the total carbon production on the earth, both land and sea combined. Steemann-Nielsen (1952), by a different method of measuring carbon fixation in sea water, arrived at a much lower rate of production, but even this lower rate would leave the production capacity of the sea equal to that of the land.

By contrast, man's total harvest of organic material from the sea is at least a whole order of magnitude lower than that from the land. Practically all of this harvest is taken from the margins of the sea. The oceanic part, beyond a depth of 1,000 m, comprises nearly 90% of the sea area, and from this major portion man's present harvest is trivial.

It is proposed in this essay to examine the means by which man may extract some of the organic wealth from this major pelagic fraction of the sea. Illustrative material will be drawn from what I believe to be the first attempt to focus scientific effort of nearly adequate scale directly on this problem. For this reason, it will be convenient to use a narrative arrangement, and consideration will be limited to the oceanic middle and low latitudes of the Pacific Ocean and to the extraction of organic materials in the form of edible fish.

The scientific effort to which I refer is the activity of the Pacific Oceanic Fishery Investigations of the U. S. Fish and Wildlife Service, headquartered at Honolulu, Hawaii, and assigned to the task of investigating the high-seas fishery potential of the tropical and subtropical mid-Pacific waters. Three vessels, the HUGH M. SMITH, the JOHN R. MANNING, and the HENRY O'MALLEY, later replaced

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by the Charles H. Gilbert, have now been operating on this program for 4½ years. The first-named has performed most of the oceanographic work while the other two have been engaged primarily in assaying the fish stocks through systematic fishing surveys.

Hawaii is located near the middle of the sluggish westward-moving North Equatorial Current. In this Current a warm upper layer overlies the colder water and forms a two-layer system of persistent stability. Typically, the warm surface layer is at least 100 m thick and has a phosphate concentration of 0.2 to 0.4 $\mu$g-at/l; zooplankton is sparse, averaging 20 ml displacement volume per 1,000 $m^3$ in the upper 200 m layer as sampled with a 0.65 mm meshed net. Such waters are obviously an unpromising environment for supporting large fish stocks unless there exist situations where the vertical stability of the two-layer system is modified so that the underlying nutrient-rich water is mixed into the lighted upper layer in greater proportion than is prevalent in the North Equatorial Current.

It was our hypothesis that in situations of such modified vertical stability there would be chemical enrichment of the euphotic zone, supporting increased biological productivity and large stocks of fish, just as is true wherever there are mechanisms for enrichment in the marginal seas flanking the continents.

Three such situations have received varying degrees of attention during the last four years: the equatorial divergence-convergence system south of Hawaii; the boundary between the North Equatorial Current and the North Pacific Drift north of Hawaii; and the perturbations within the North Equatorial Current as it flows past or through the Hawaiian Islands group. Each of these involves a different physical mechanism for energy transformation into vertical motion: upwelling in the first situation; current interface shearing in the second; and eddies and wakes in the third. All of these situations appear effective for producing important concentrations of fish. Our knowledge regarding the first is now considerable. It is only fragmentary for the second and third. Even in the first situation there remain large areas of ignorance in our understanding of the physical and biological phenomena involved.

At the outset of our investigation, upwelling along the Equator, as recorded in the data of the Carnegie expedition of 1928–1929 and as interpreted by Sverdrup, *et al.* (1942; 710), appeared to be the most promising large-scale mechanism for producing concentrations of fish. Here, some kinds of fish should be abundant if our hypothesis was valid. It was hoped that these would include the two tropical and subtropical species of tuna that are of greatest commercial importance, namely the yellowfin tuna, *Neothunnus* *macropterus* Temminck and Schlegel, and the skipjack, *Katwuwonus pelamis* Linnaeus.

Accordingly we undertook an intensive study of the equatorial current system. It has now been under way for four years. In this period we have made 16 hydrographic traverses of the current system at various meridians from Long. 140° to 180° W. On all of these traverses, bathythermograph casts were added between stations, usually at 10-mile intervals, and about 30 additional BT sections were made on fishing voyages. These extend our knowledge of the detailed temperature field down to 900 feet as far east as Long. 110° W. Several of the hydrographic traverses extended to Lat. 24° N and one to 14° S. However, the rectangle between Long. 140° and 180° W and Lat. 12° N and 10° S has been the most thoroughly explored. On many of the traverses, zooplankton hauls were made with a conventional meter-net, hauled obliquely from the surface to 200 m, with the flow through the net measured by a current meter.

These observations proved that there was indeed an upward movement of water at the Equator (Cromwell, 1951) and that it was accompanied by a greater concentration of zooplankton (King and Demond, 1953) in the vicinity of the Equator than in adjacent higher latitudes.

Despite the consistent evidence of chemical enrichment accompanied by zooplankton abundance, extensive fishing trials made by the three principal methods used in the California commercial tuna fishery (live-bait chumming, purse-seining, and trolling) proved disappointing. Except for small areas in the immediate vicinity of the few low-latitude islands in the region, tuna were not caught in the zonal band of enrichment.

Faced with this contradiction, we employed a fourth fishing method heretofore used only by the Japanese. Known as the "longline," this consists of a mainline suspended from surface buoys at intervals and provided with baited hooks hung by dropper lines at intervals along the mainline. This gear is constructed in units, known as "baskets," which are joined end to end and which may extend many miles. In our survey fishing, 12 miles of line are set, with the buoyline lengths and the slack in the mainline so adjusted that the hooks fish at various levels from 100 to 400 feet, or for all practical purposes, throughout the entire warm layer and down partly into the transition layer.

This method of fishing proved to be satisfactory for sampling the larger pelagic carnivores and gave results consistent with the oceanographic findings. It has now been employed to delineate the distribution of tunas over a rectangle of about 4,000,000 square miles

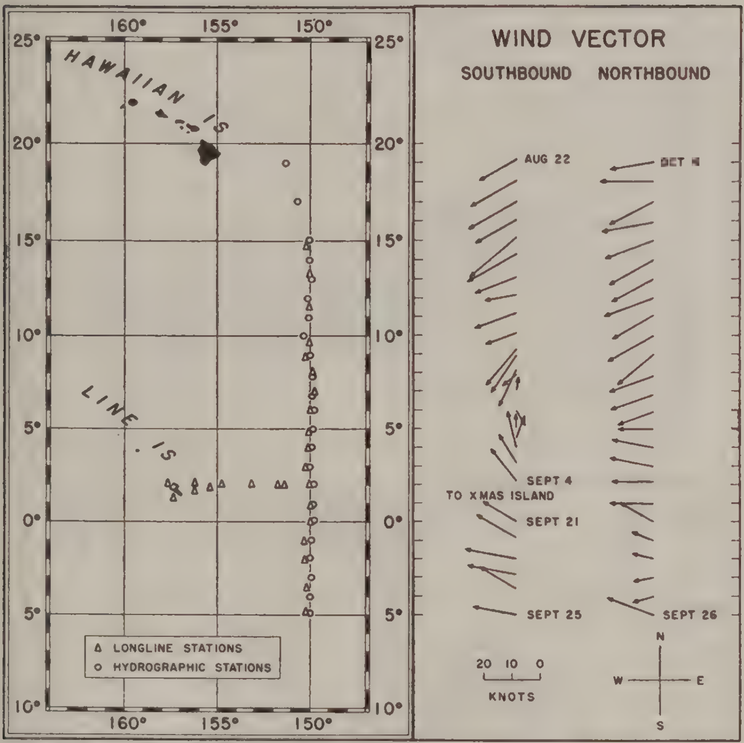
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Figure 1. Hydrographic and fishing stations occupied by the Hugh M. Smith from August 22 to September 26, 1951 (left) and the average direction and force of the wind at intervals along her track (right).

from Lat. 10° N to 5° S and from Long. 110° to 180° W. Further, the efficiency of this gear, first thought to be too low for use in the American economic structure, has proved sufficiently high to support American commercial operations when such gear is used in the areas of the greatest concentration of tuna as delineated by our surveys.

The findings with this sampling instrument in relation to the equatorial current system may be illustrated by an example along Long. 150° W. During her Cruise 11 in August and September 1951, the Hugh M. Smith combined hydrographic, plankton, and longline operations along the track shown in Fig. 1. On the southbound traverse the winds were nearly typical for the region: northeast trade

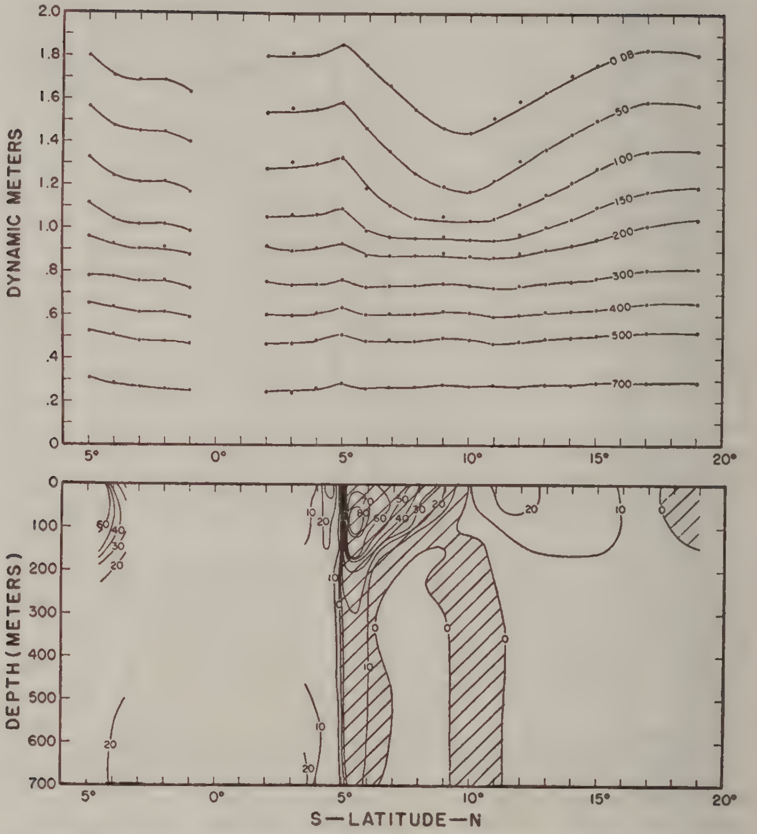

Figure 2. Dynamic topography (upper) and field of horizontal velocity (lower) inferred from geostrophic analysis of hydrographic data taken along the 150th W meridian by the Hugh M. Smith, August 22 to September 25, 1951. The unshaded portion represents flow to the west, the shaded portion flow to the east.

winds in the northerly portion of the traverse, southeast trade winds in the southerly portion, with light and variable winds between Lat. 5° and 9° N in a doldrum belt that separates the two trade wind systems. On the northbound traverse a month later, the doldrum belt was not in evidence, easterlies prevailed in the south, and the transition to northeast trade winds at 5° N was immediate. Geostrophic analysis (Fig. 2) of data from the northbound traverse

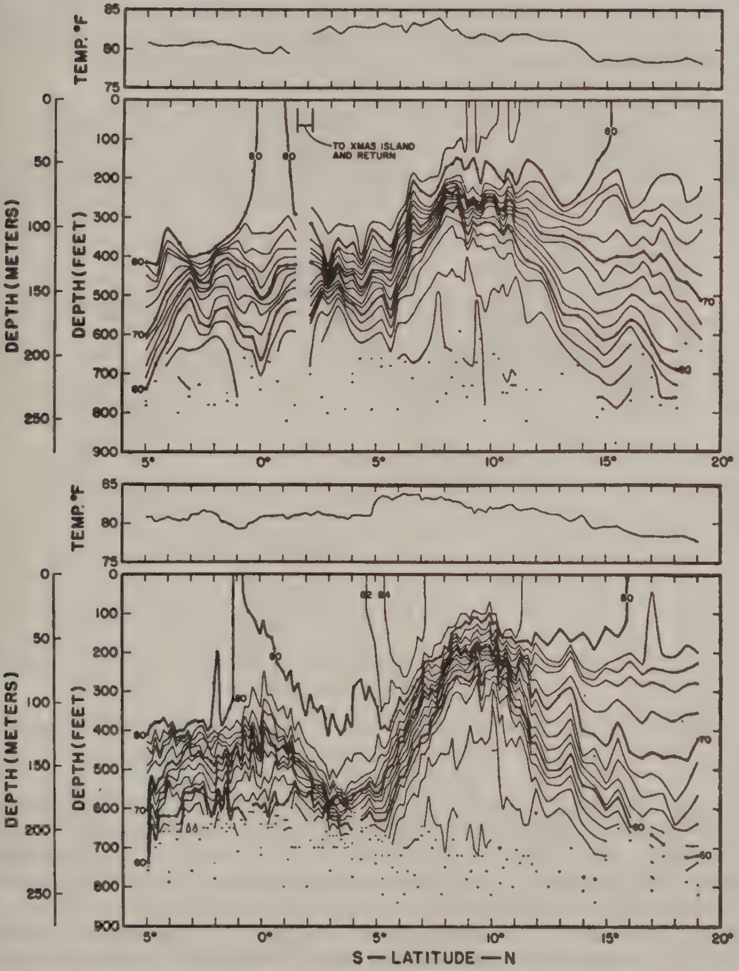

Figure 3. Vertical fields of temperature as measured by bathythermograph on traverses along the 150th W meridian: August 22 to September 25, 1951, southbound (upper) and September 26 to October 4, 1951, northbound (lower).

places the Countercurrent in its usual position between Lat. 5° and 9° N with a strong eastward flow of 80 cm/sec at its core. The drift of longline (Fig. 7) confirms this interpretation and also betrays the strong easterly undercurrent at the Equator, recently described by Cromwell, *et al.* (1954).

The transverse circulation is suggested by the distribution of properties in cross-section. Temperature (Fig. 3), density (Fig. 4), and inorganic phosphate (Fig. 5) show a weakening of stability at the Equator. The isopleths for 80°F, for $\sigma_t$23.0, for 0.6 $\mu$g-at $PO_4$-P/l and for 4.5 ml $O_2$/l ascend nearly vertically to the surface at or near the Equator, indicating relatively freer vertical exchange through the upper part of the water column at the Equator than elsewhere along the traverse. The isopleths immediately below those mentioned bend upward. This suggests that the exchange involves at least part of the underlying transition layer. In the deeper layers they dip downward under the Equator. Whether this is indicative of still deeper exchange is problematical.

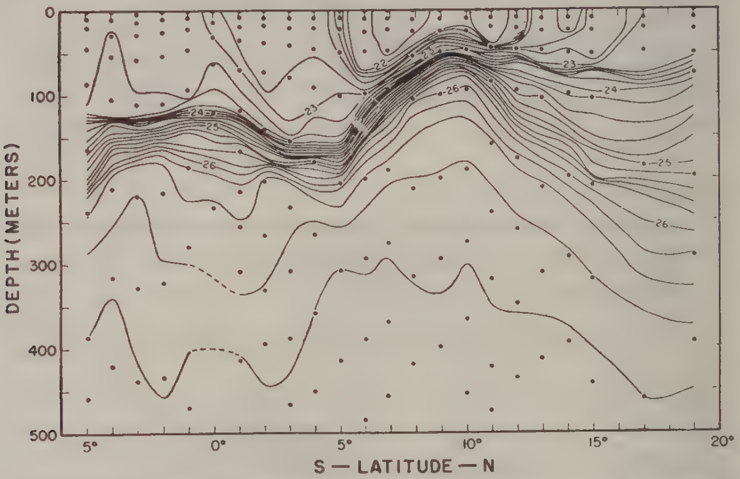

**Figure 4.** Vertical field of density along 150th W meridian, September 26 to October 4, 1951.

It is not my intention here to elucidate fully the dynamics of this transverse equatorial circulation. As already suggested, there are features of it which are only partially understood and others are not understood at all. However, Cromwell (1953) has offered a model,

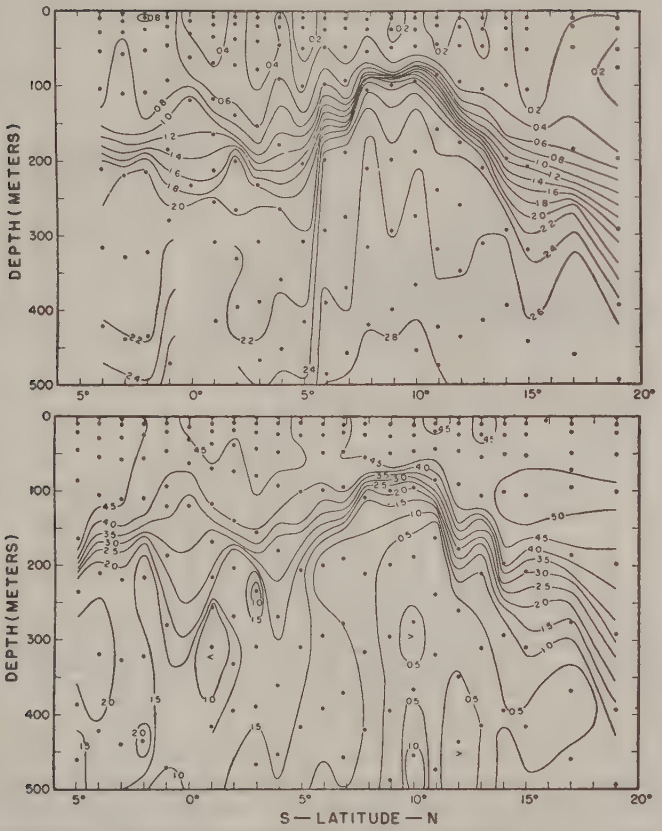

Figure 5. Inorganic phosphate in microgram atoms per liter (upper) and oxygen in milliliters per liter (lower) along the 150th W meridian, September 26 to October 4, 1951.

based on isentropic principles, which relates the transverse circulation to the winds and accounts for the major features we have observed in the distribution of properties in cross-section (Cromwell, 1954; Austin, 1954). We have reproduced in Fig. 6 the diagram of the transverse circulation as published by Cromwell (1953: Fig. 7). Briefly, the stress of easterly winds impels the surface water in a westerly direction at the Equator, but the Coriolis force north and south of the Equator tends to deflect the surface water poleward away from the Equator, where deeper water rises toward the surface.

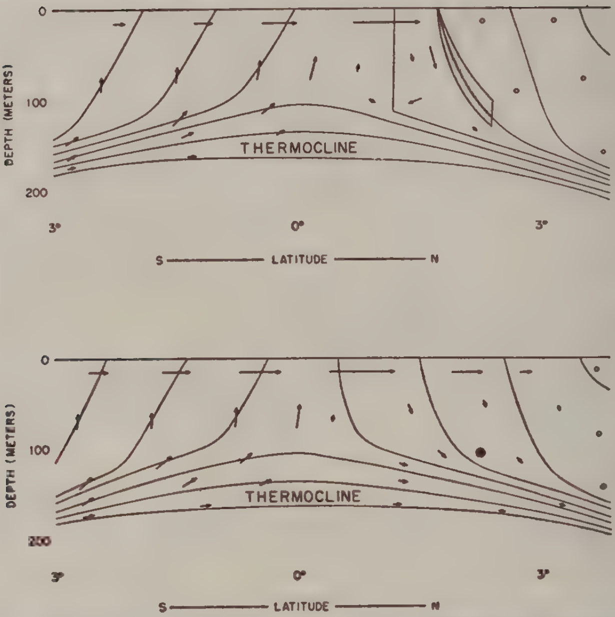

Figure 6. Schematic representation of circulation in the meridional plane near the Equator during a southeast wind when a front is present (upper) and when no front is present (lower). Lines represent surfaces of constant density. Arrows have the character of streamlines, not trajectories, and their shafts indicate roughly the relative magnitudes of flow. After Cromwell (1953: fig. 7).

Asymmetry is introduced when there are northerly or southerly components in the easterly wind. For instance, according to Cromwell, southeast trade winds displace the center of the upwelling to the south of the Equator. This appears to have been the condition during Cruise 11 of the HUGH M. SMITH.

North of the Equator the upwelled water probably sinks, forming a zone of convergence between the center of the upwelling and the southern edge of the Countercurrent. Fronts are sometimes present in this convergence zone. The sinking water may return southward at deeper levels, thus completing a cell, or it may continue northward, diving under the Countercurrent. In the latter event, associated movement in other directions would be required by the principle of continuity.

When the zooplankton and fish catches along Long. 150° W are compared with the temperature section (Fig. 7), it is seen that the

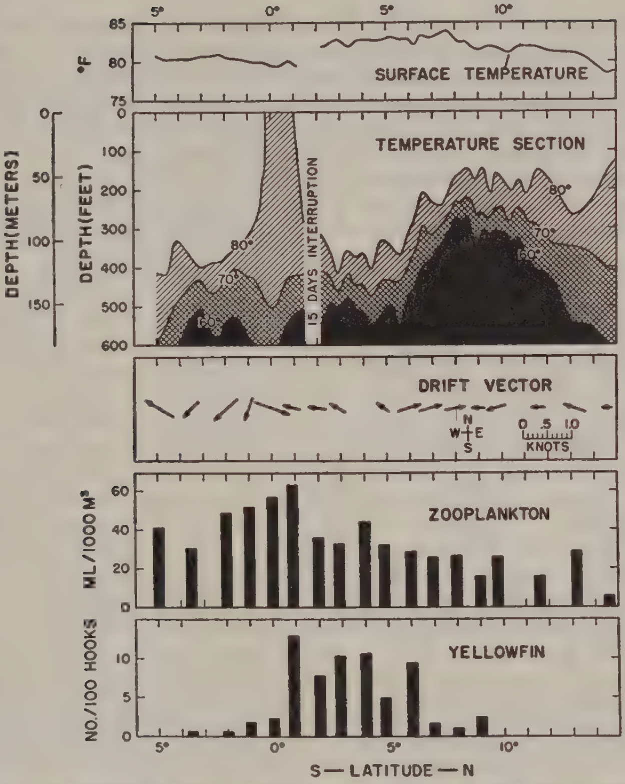

Figure 7. Surface temperature, vertical temperature field, longline drift, zooplankton abundance in the upper 200 m, and the yellowfin tuna catch along Long. 150° W, August 22 to September 25, 1951.

plankton concentrations are high in the vicinity of the Equator and that the tuna concentration is high in the zone of convergence. The displacement of the tuna northward from the plankton maximum is not unexpected since at least one trophic level (small fishes, crustacea, and squids) intervenes in the food chain.[1]

True upwelling seems to be absent at the northern boundary of the Countercurrent, though the transition layer here ascends so near the surface that a brief[2] invasion of the doldrum belt by high winds might stir the deeper, cooler, richer waters into the surface layer.[3] This evidently does not occur regularly enough to establish a trophic succession culminating with large fish, for we have not found a zone of yellowfin tuna concentration in or along the northern boundary of the Countercurrent, nor indeed in any part of the Countercurrent.

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[1] A speculative discussion of this displacement and also of the lack of a comparable tuna concentration south of the Equator was given at the 8th Pacific Science Congress (Sette, in press).

However, in the convergent zone area between the Equator and the Countercurrent, or sometimes in a band centering at the Equator, we have never failed to find a zone of much better longline tuna fishing than at adjacent higher latitudes. The quantities of catch and the positions of the zone in the north-south direction have varied considerably,[4] probably in response to accelerations and decelerations as well as to the swaying north and south of the current system. Although our observations have not had sufficient continuity in time and space to elucidate these variations, it remains quite clear that the divergence-convergence features of the transverse equatorial circulation provide the basic support for a persistent concentrated stock of yellowfin tuna.

The yellowfin tuna sampled by our survey are the older, larger individuals of the species, ranging from 60 to 180 lbs. and averaging about 100 lbs. The younger members of the species are nearly absent from our longline catches. So also are the skipjack. In our equatorial plankton hauls, however, the yellowfin tuna larvae occur regularly, and the skipjack larvae outnumber the yellowfin larvae by about three to one (Fig. 8). Obviously, equatorial waters must harbor young yellowfin in greater quantities than older ones (to allow for mortality), and in addition they must support still greater numbers of skipjack. A truly enormous oceanic fishery may be envisioned when the full potentials of this area are realized.

The east-west extent of the equatorial stock is limited only by the two continents, which at the Equator lie 10,000 miles apart. To be sure, this entire extent does not enjoy the high concentration we have found south of Hawaii. This concentration seems to be the consequence of the unequal stress of the easterly winds along the Equator. According to Cromwell's model, the upwelling should occur along

[2] The probably short-lived invasion by moderate northeast winds during the northbound traverse of the Hugh M. Smith apparently was without discernible effect on the vertical distribution of phosphate.

[3] This may have happened sometime prior to July 1952, for in that month unusually high plankton catches were taken in and immediately north of the Countercurrent along Long. 140° W. This is the only instance of such catches among the many zooplankton sections we have made across the Countercurrent.

[4] Some examples are given by Murphy and Shomura (1953).

the entire extent of the Equator lying under easterly winds, but it should be strongest and the enrichment of surface waters should be greatest where the winds are strongest and most sustained. According to Pilot Charts, the average wind force is maximal at about Long. 120° W. Therefore, the greatest enrichment should occur on the average at about the 120th meridian. If the two phenomena, maximal enrichment and maximal tuna concentration, are in cause and effect relationship to each other, then the westward flow of the water concurrently with the passage of the biota through at least three trophic levels (phytoplankton, zooplankton, and smaller nekton) should have rates in reasonable relation to the distance involved. A parcel of water travelling westward from Long. 120° to 150° W at the rate of ½ to 1 knot (an estimate based on drift of longline in the convergence zone just north of the Equator) would be carried 1,800 miles to the zone of best tuna fishing in about 75 to 150 days. Whether or not this is a reasonable period of time for the three trophic levels of development is a speculative question. It does not appear to be fantastic, however, and it is the most cogent explanation I can offer for the special concentration of tuna at Long. 150° to 170° W (Fig. 9).

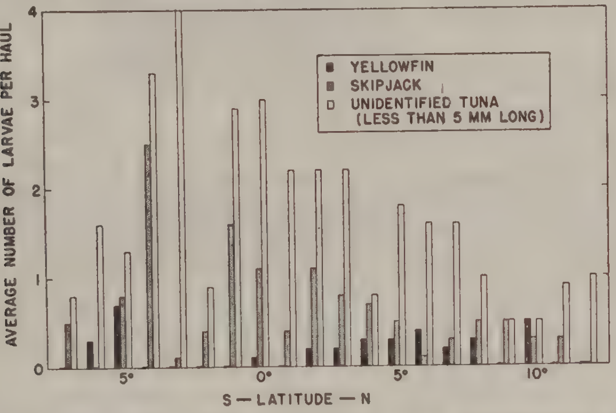

Figure 8. Average numbers of tuna larvae taken per oblique tow of 30 minutes duration through the upper 200 m layer with a one-meter tow-net having 0.65 mm mesh apertures. The samples were collected along various longitudes from 140° to 180° W and in various months during the period from June 1950 to July 1952. Larvae under 5 mm in length were not identified as to species.

This concentration of tuna directly south of Hawaii is now undergoing development as a commercial tuna fishery resource by American fishermen. The lesser concentrations of yellowfin tuna lying west

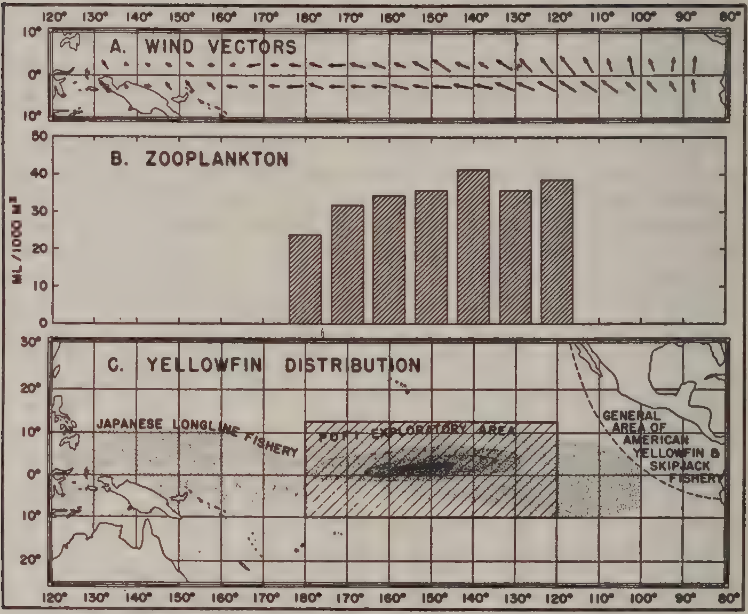

Figure 9. Wind, plankton, and tuna distribution in the equatorial zone. *A.* Resultant wind vectors were computed as the weighted means of frequency and speed from the several compass points for 5-degree squares bordering the Equator as published in the U. S. Hydrographic Office Pilot Chart for the North Pacific for August 1952; the arrows point in the direction of travel and their shafts are propertional to the weighted mean speed. *B.* Mean plankton volumes at several longitudes were computed as the mean of all hauls taken between Lat. 5° S and 10° N at or near the given longitude. *C.* Distribution of yellowfin tuna in the mid-Pacific equatorial region is estimated from longline catches. The light stippling in the POFI area represents less than three yellowfin tuna per hundred hooks, the heaviest represents more than nine per hundred hooks, and the intermediate stippling represents intermediate catching rates. For orientation as to location and area, the extensive band of light stippling represents the area of the Japanese prewar longline fishery; the dashed outline shows the area of the American west coast live bait and purse seine fishery for yellowfin and skipjack tuna. Since preparation of the above chart, a line of fishing stations along Long. 110° W has proved the continuity of the band of yellowfin tuna across the "unexplored area."

of the 180th meridian are already commercially useable in the Japanese economy. The lesser concentration to the east of Long. 150° W should ultimately be useable in the American economy when oceanographic advances make the finding of local concentrations within the convergence zone more sure and when technological advances make the fishing methods more efficient. It is perhaps not overly optimistic to say that a vast oceanic fishery resource is well on the way toward full utilization.

This initial success of our hypothesis as a working tool for discovering fishery potentials in the oceanic two-layer system encouraged us to extend this type of approach in research to the second situation, the shear zone between the North Equatorial Current and the North Pacific Drift. In this more northerly latitude a cool-water tuna, the albacore, *Germo alalunga* (Bonnaterre), may be expected. Indeed, the Japanese have already developed a fishery for this species in the western Pacific between Lat. 30° and 40° N and between their home islands and the longitude of Midway Island some 2,500 miles eastward. Uda (1953) has attributed this fishery to a feature he designates as the Subtropical Convergence.

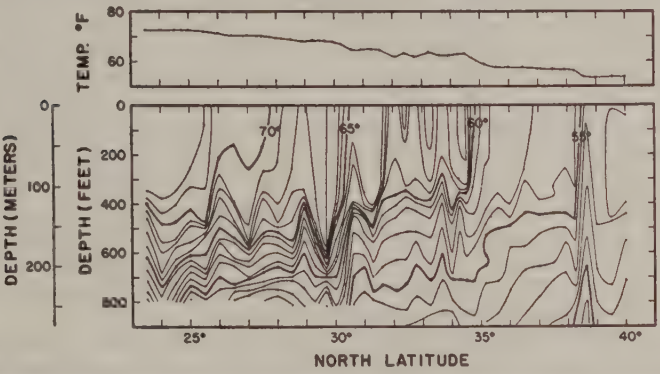

Figure 10. The vertical field of temperature as drawn from uncorrected bathythermograms along Long. 160° W in January 1954.

Our first reconnaissance north of Hawaii in January through March 1954 crossed the North Equatorial Current into the North Pacific Drift along 10 meridional lines between Long. 140° and 165° W. The data from these crossings are not yet processed.[5] A preliminary temperature section (Fig. 10), drawn from uncorrected bathythermograph recordings, suggests a broad zone of complex thermal structure with most pronounced transitional features at Lat. 34° N. In several instances the bunching of isotherms in their vertical ascent from the transition layer to the sea surface may result from eddy fragmentation in the shear zone. Their significance to vertical exchange must await further study. Between Lat. 30° and 35° N the longline fishing gave evidence of abundant fish life, including bigeye tuna, *Parathunnus sibi* Temminck and Schlegel, a species presently not considered a good canning variety, sharks, lancet fish (*Alepisaurus* sp.), and miscellaneous others including what is probably a new species of pelagic sting ray. At 34° on this section an outstanding catch of albacore was made. It seems quite likely, therefore, that we have here a general locality where the second type of vertical circulation provides the potentials of a fishery.

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[5] The fishery research vessel N. B. SCOFIELD of the California Department of Fish and Game, in cooperation with the Scripps Institution of Oceanooraphy, made four more meridional sections between 140° W. longitude and the American west coast.

The third mechanism, which concerns vertical circulation of deeper waters into the euphotic zone within the North Equatorial Current system, should occur where islands interrupt the current's flow. This is suggested by the existence of a small but productive skipjack fishery in Hawaii. After vainly searching for enriching mechanisms connected with perturbations in the current flow past these islands, we recognized last summer the presence of relatively large-scale and semipermanent eddies downstream from the Hawaiian Islands. Curiously enough, these were recorded in the very first oceanographic observations taken by the HUGH M. SMITH in the course of her shakedown trip in December 1949. They were first recognized however by GEK observations taken last summer. Both convergent and divergent types of eddies are formed (Fig. 11).

Near the boundary of one of the divergent eddies and far beyond the range of the present small local Hawaiian skipjack fishery, systematic scouting operations recorded many more skipjack schools than were sighted in the near-shore grounds or in neighboring offshore waters (Royce and Otsu, MS). This concentration was found on two occasions. Both times it was near the western edge of the most pronounced divergent eddy. This suggests that the eddy system has within it a train of physical and biological events of significance to the support of the fish. Also, the finding of these concentrations well outside the range of the present fishery suggests further that the productivity of this middle portion of the North Equatorial Current has potentials far beyond those suggested by the existing small local fishery.

Thus it appears that there are three situations in oceanic waters where the two-layer system is sufficiently modified to permit the vertical exchange necessary for supporting commercial fish stocks. Others may become apparent as our knowledge increases.

In comparison with the fisheries of marginal seas, the utilization of such oceanic fishery resources affords special opportunity for

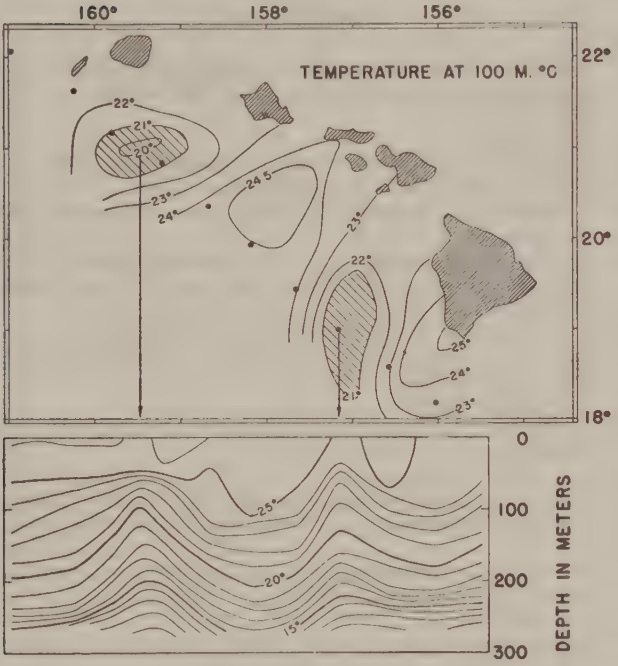

Figure 11. Temperature at the 100-m level (upper) and in a vertical section passing through the black dots of the upper diagram (lower) southeast of the Hawaiian Archipelago in August 1954.

oceanographic research to be of signal service. Unlike the fisheries of marginal seas, which were developed essentially by following topographic features to their seaward limits, the development of oceanic fisheries requires launching out beyond topographical landmarks literally into the deep blue. Success in locating fish resources in the deep blue does not reward the hit or miss method. Past ventures in the hands of so-called practical fishermen have proved this for the oceanic Pacific. At least four such ventures, incurring millions of dollars of expenditure, took place during the five years preceding the commencement of our work. None was successful. Fishermen's lore, evolved in the topographical setting of marginal seas, is not effective for guidance in oceanic fishing. Oceanographic research, on the other hand, promises to be effective in providing the needed guidance.

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*Studies on parasites of the green crab, Carcinides maenas.* HORACE W. STUNKARD.

Since *Carcinides maenas* is a serious predator of *Mya arenaria*, the U. S. Fish and Wildlife Service is interested in determining whether or not its parasites can serve as possible means of biological control. It has long been known that *C. maenas* in the Woods Hole area is infected by an undetermined, encysted metacercaria and experiments have been conducted to discover its identity, life-history and biology. Metacercariae were excysted to study their morphology and cysts were fed to recently hatched, uninfected birds, *Sterna hirundo* and *Larus argentatus*. Large numbers of worms were recovered, including all stages from juvenile to fully mature specimens. The structure of the metacercariae suggested that they may be specifically identical with a minute, stylet-bearing cercaria which occurs in *Littorina obtusata*, *Littorina saxatilis*, and rarely in *Littorina littorea*. Small green crabs exposed to these cercariae became heavily infected; enormous numbers of worms entered the tissues and developed to metacercariae, identical with those of the natural infections mentioned above. Small crabs exposed continuously with six to eight infected snails died in ten to twenty days, and on dissection yielded thousands of larvae. The stages in the life-cycle of the parasite agree with descriptions by European investigators of corresponding stages: the metacercariae with metacercariae from *C. maenas*, described but not named by M'Intosh (1865); the adults with *Microphallus similis* from Swedish gulls, described and named by Jägerskiöld (1900); and the cercariae with *Cercaria ubiquita* Lebour, 1907. The identity of these worms and their relation as stages in the life-cycle of a single species is predicated. It is possible that the parasites and their intermediate hosts are introduced species.

# CONSERVATION OF AN EAST COAST SHAD FISHERY

G. B. TALBOT

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# Conservation of an East Coast Shad Fishery

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MOST FISHERY ADMINISTRATORS and biologists would agree that *Conservation* relating to fishes means to them something like "wise utilization of fishery resources." This, of course, implies some sort of management of the fishery. How this is to be accomplished, or indeed, even if it can be, does not seem to be so readily agreed upon.

Usually a fishery is depleted before critical management procedures are considered, and corrective measures then call for restriction of the catch, at least during the initial stages of recovery. Huntsman (1953), however, has stated, "There seems to be no good basis for restricting fishing in order to increase the long term yield." Taylor (1951) has also pointed out that although "particular species have fluctuated in abundance, the yield of the sea fisheries as a whole or of any considerable region has not only been sustained, but has generally increased . . ." Others have argued (Burkenroad, 1951) that natural fluctuations in abundance may be the cause of the declines in some fisheries and that little can be done in controlling them.

On the other hand, where serious attempts have been made to understand the dynamics of a fish population and then to manage the fishery on scientific principles, the results have been most encouraging. Outstanding examples on our west coast are the work on Pacific halibut which has been notably successful, and on Fraser River sockeye, which has been a spectacular success.

In 1950 the Atlantic States Marine Fisheries Commission sponsored legislation to provide funds to the Fish and Wildlife Service for a coast-wide study of the shad *(Alosa sapidissima)* in order to provide basic information for state shad fishery regulations along the Atlantic Coast. As part of that study the Service began its work with investigation of the Hudson River shad. Emphasis was placed on defining the dynamics of the fishery to the extent that the possibility of managing the fishery could be explored. The Hudson fishery was of particular interest, since, contrary to the general downward trend of the Atlantic shad fishery since the beginning of the century, the catch had increased between 1936 and 1946 to almost its former level of production, and then declined to a low level by 1950. The objectives of the study were to discover the factors causing the decline, to determine conditions favoring recovery, and to provide information for proper management of the fishery. This study has been completed (Talbot, 1954) and the fishery is now being managed with excellent results.

When work was begun on the Hudson River it was realized that the catch made each year by the fishermen was not necessarily a good index of the size of run since the size of the catch depends not only upon the size of run, but also upon the number of nets being fished. To establish the relationship between the catch and size of run (fishing rate) a tagging program was carried out.

In 1951, 524 shad were caught, tagged and released at the mouth of the Hudson while the shad run was in progress. These mixed with the untagged shad and continued upstream with them. They were first intercepted by the New Jersey fishermen who fish near the mouth of the river and then by the New York fishermen who fish farther upstream above the state boundary. These fishermen were contacted regularly and their total catches were recorded along with the number of tags they recovered. A total of 755,000 pounds of shad were caught along with 46 per cent of the tagged fish. Since 46 per cent of the tagged fish were caught, we could infer that 46 per cent of the shad in the river were caught and, therefore, by simple proportions the total run entering the river was calculated to be 1,641,000 pounds of shad.

A total of 1,641,000 pounds was, therefore, available to the New Jersey fishermen and, according to our records, they had caught 332,000 pounds. There was then 1,641,000 pounds minus the 332,000 pounds caught, or 1,309,000 pounds available to the New York fishermen. Of this amount the New York fishermen caught 423,000 pounds, leaving 886,000 pounds of shad to spawn.

Statistics showing the catch of shad in the Hudson River by New York and New Jersey fishermen, and also the number of nets used each year, were available for each year since 1915. The New Jersey gear appeared to be much more efficient than the New York gear—probably because the river is narrower than in the New York section. It was, therefore, necessary to determine the fishing power of the gear of each state so that they could be combined to determine the total fishing effort for each year. To do this the following calculations were used: We let $p$ equal the fishing power of a single unit of gear, and defined "fishing power" to mean the ability of one unit of gear to capture a certain fraction of the fish present in the river in one day's fishing. We let $q$ equal the fraction of the fish that were not captured so that $p + q = 1$. Now, if we consider the run as a whole and denote its size by $N$, the number of pounds of shad removed in one net-day of fishing would equal $pN$, and the number remaining would equal $qN$. The fish not caught by the first net-day of fishing are susceptible of being captured by the second net-day of fishing during which $pqN$ are removed. The number of pounds removed and the number of pounds remaining after successive net-days of fishing would then be as follows:

| | No. of pounds removed | No. of pounds remaining |
|---|---|---|
| 1st net-day | $pN$ | $qN$ |
| 2nd net-day | $pqN$ | $q^2N$ |
| 3rd net-day | $pq^2N$ | $q^3N$ |
| 4th net-day | $pq^3N$ | $q^4N$ |
| * | * | * |
| * | * | * |
| * | * | * |
| n-1 net-day | $pq^{n-2}N$ | $q^{n-1}N$ |
| nth net-day | $pq^{n-1}N$ | $q^nN$ |

After the nth net-day of fishing there remains $q^nN$ pounds of shad in the river.

In this case n equals the fishing effort, or in other words, the number of nets fished multiplied by the number of days they fished which was available; and N, the total run, and the number of pounds of fish remaining (spawning escapement) were determined from the tagging program. The only unknown, therefore, is q which can be calculated for the gear fishing in each state. From the calculated q's, the p's were determined easily, since $p = 1\text{-}q$. We then had a measure of the fishing power for the gear of each state and found that the New Jersey gill-nets had about five times the fishing power of the New York nets. A New York net was taken as the standard unit of fishing effort and the total net-days fished each year between 1915 and 1951 by the New York fishermen were combined with net-days fished each year by the New Jersey fishermen, increased five-fold. This gave us the total fishing effort exerted by the shad fishermen of the Hudson River for each year since 1915 in standard fishing unit days.

Now, as previously shown, pN is the number of pounds of shad removed the first standard fishing unit day, pqN is the number of pounds removed the second fishing unit day, and so forth. It can be seen that in any season the total catch is as follows:

$$C = pN + pqN + pq^2N + pq^3N + \ldots\ldots\ldots\ldots + pq^{n-1}N$$

Factoring,

$$C = pN\ (1 + q + q^2 + q^3 + \ldots\ldots + q^{n-1})$$

Since the expression in the parenthesis is a geometric progression, its sum can be expressed by the formula $\frac{1-q^n}{1-q}$

Substituting, we have:

$$C = pN\ \frac{(1-q^n)}{(1-q\ )} \text{ and transposing}$$

$$N = \frac{C}{p\frac{(1-q^n)}{(1-q\ )}} \text{ or, since } p = 1-q,\ N = \frac{C}{1-q^n}$$

Since we knew the total catch (C) made in the river each year since 1915, and had the total fishing effort, n, for each year and also had the value, q, it was now possible to calculate the total run of shad, N, for each year since 1915. Since we also had the catch for each year we could, by subtraction, find the amount of shad escaping the fishery and spawning each year. With this information it was possible to compare fluctuations in size of run with factors suspected of influencing the size of run. It was not possible to do this with only the catches of shad made each year because they are influenced by the amount of gear used in catching the fish.

Many factors were suggested by persons connected with the Hudson shad fishery as being responsible for the recent decline. These were: changes in stream flow, changes in water temperature, dredging to improve the ship channel, increase in ship traffic, curtailment of hatchery operations, pollution, natural cycles of abundance and scarcity, catches made outside the river, and overfishing. All these things were considered during our investigations.

However, before we could determine cause and effect relationship with any of these factors, additional information was needed concerning the biology of the shad. We knew that the adults return to the river of their origin in the spring of the year for the purpose of spawning, and it is at this time that the commercial fishery takes the harvest. Those that escape the fishery and do not die from natural causes, spawn and return to the ocean, and if they do not die of natural causes or are not caught elsewhere, they come back again the following spring to spawn. The eggs of the shad hatch in five or six days, and the young live in the river until the fall when, at a length of three or four inches, they leave for the ocean. It was vitally necessary, however, to know at what age these fish return as adults to spawn for the first time, since any factor adversely affecting the young, for instance, would not become evident in the fishery until the time they would normally return as adults.

To determine this, scales from a sample of shad were taken throughout the runs of 1950 and 1951 from the catches made by the fishermen. A method was found to read these for total age as well as for the number of times they had previously spawned. It was found that the majority of shad returned to the river as adults for the first time at four and five years of age. Therefore, it could be expected that any factor affecting the young of any one year would not show until four and five years later. It was also found from the scale readings that about 50 per cent of those captured had spawned in previous years.

With the age-composition of the shad known, it was possible to make valid comparisons between factors suspected of affecting the run and the size of run each year.

No effects from variations in stream flow or water temperatures could be found. Dredging in the river had no measurable effect on size of shad run, but deepening the river increased the number of ships running up the river to Albany. Shipping appeared to have no effect on the size of shad run, but did increase the hazards of fishing and caused loss of fishing gear and damage to boats.

Production of shad hatcheries had no measurable effect on subsequent size of the shad runs and no evidence of natural cycles of abundance was found. Evidence of gross pollution of the river was found, but this condition has apparently been serious since the beginning of the century. Pollution abatement probably would increase the productivity of the river and certainly would result in a higher quality fish, but there was not enough evidence available to compare fluctuations in the size of run with changes in pollution. Catches outside the river undoubtedly influenced the run, but there was no way to distinguish the Hudson River fish from the other shad caught outside the river and so this effect could not be determined.

In analyzing the effect of fishing on the size of the run, or in other words, to determine if overfishing caused the recent decline, a comparison was made between the size of run in any year and the size of the spawning escapement the year previous and four and five years previous. This was done because it had been shown from the scale readings that about 50 per cent of the catch was made up of shad which has escaped the fishery the year previous, and that the majority of the fish caught were four and five years old. Those allowed to escape the fishery and spawn would return the next year in the fishery and their progeny would return and enter the fishery mostly four and five years later. This comparison was made using a mathematical analysis termed a multiple regression analysis. This disclosed that there was a very significant correlation between the amount of shad allowed to spawn each year and subsequent shad runs. In fact, 85 per cent of the fluctuations in size of run could be accounted for by changes in the escapement. Since the size of run is

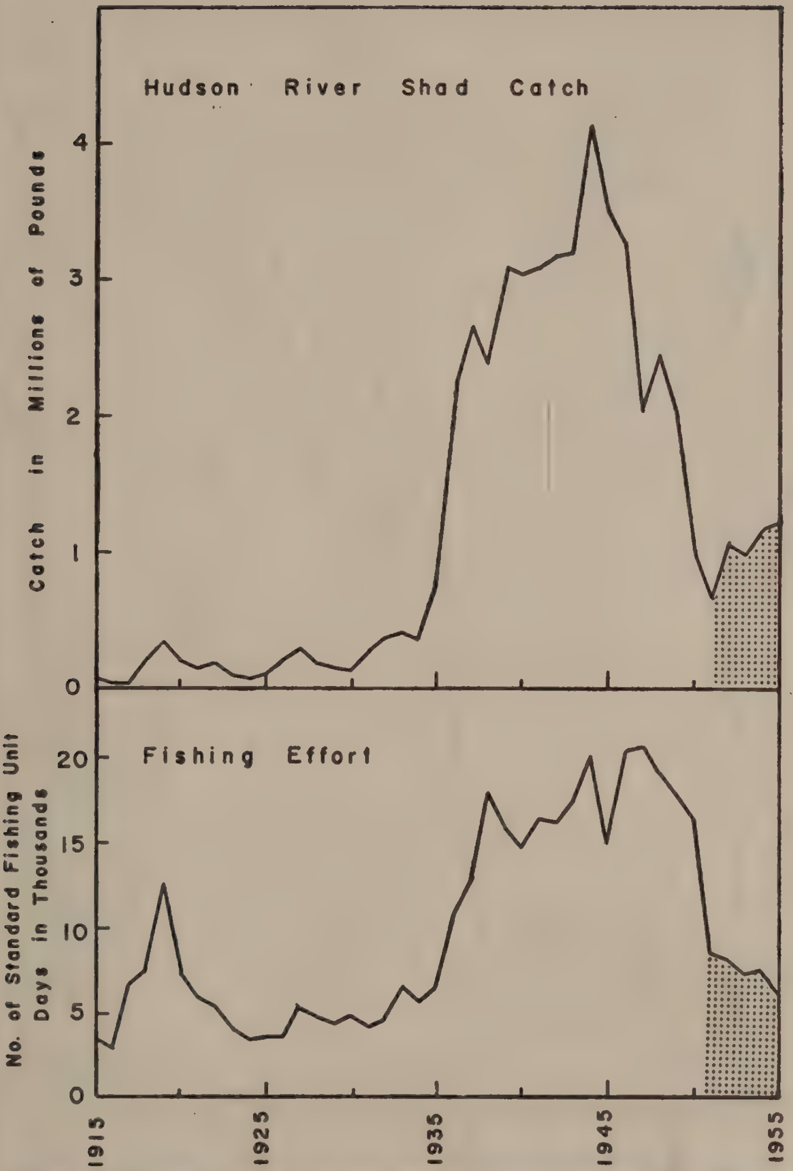

FIGURE 1.—Hudson River shad catches and fishing effort from 1951 through 1955. Data to 1951 from Talbot (1954). Data after 1951 from New York and New Jersey statistics.

determined by the amount of fish allowed to spawn, it was possible to predict by the use of an equation, using the calculated escapements what the total run should be one year in advance. This was done for each year for

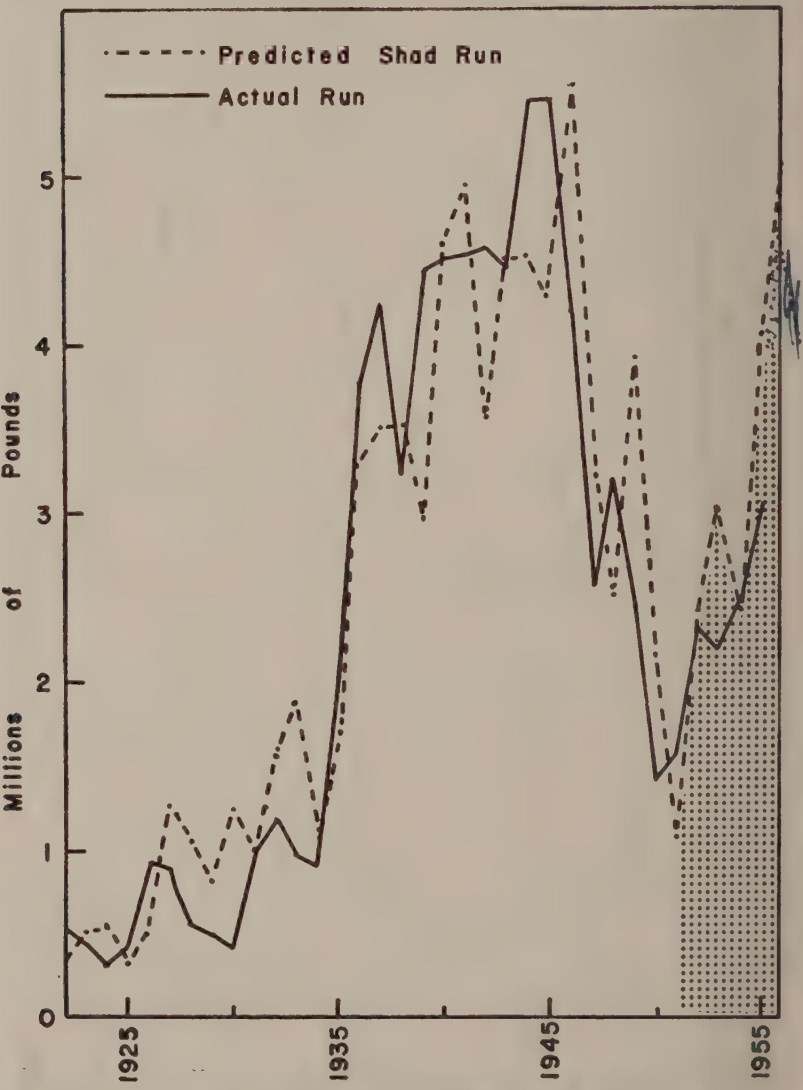

FIGURE 2.—Predicted and calculated actual shad runs, in pounds for Hudson River, 1922 through 1956.

which statistics were available and the predicted populations agreed closely with the actual calculated populations. It could be seen from these comparisons that the amount of fish allowed to escape the fishery each year was responsible not only for the recent decline, but also for the great increase in size of run between 1936 and 1946. The equation, plus the statistics of the catch and effort, were the tools needed to regulate the fishery to produce maximum yields each year. Since the size of run could be predicted one year in advance, fishery regulations could be formulated to allow a predetermined escapement which would give the desired size of run four and five years later.

In 1951 it was realized that the fishing intensity was too great for the size of shad runs, and concurrent restrictive regulations of the fishery were imposed by both New York and New Jersey as a means of conserving the fishery. The fishing intensity was decreased to about one half that which had been in effect for the past several years and in 1951 only 755,000 pounds were caught. The small sacrifice in catch, however, has paid off. The lower catch resulted in an increased spawning escapement that year and the following year the shad run began to increase because of the increased "repeaters" from the year before. The fishing effort has continued to decline, but in spite of less fishing being done, the total catch of shad has increased from 755,000 pounds in 1951 to over 1,200,000 pounds in 1955 (Fig. 1), and in 1955 the total run was over 3 million pounds as compared to half that amount in 1950 and 1951 (Fig. 2). As a result of the facts developed by the intensive study of the Hudson River shad and the prompt action of the States of New York and New Jersey in amending their regulations, this fishery is now well on its way toward being restored to its former level of abundance.

The results of this study appear to add weight to the theory that at least some of our fishery resources can be scientifically managed so as to produce maximum sustained yields year after year. In many cases the problems involved in doing this are not great. The most important tools necessary are adequate catch and effort statistics compiled over a series of years. These, in combination with a comprehensive research program, can point the way to sound conservation programs.

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# Recent Equatorial TUNA RESEARCH

Reprinted from Pan American Fisherman, March, 1956

# *Recent Equatorial* TUNA RESEARCH

**By ALBERT L. TESTER**

*Director,*
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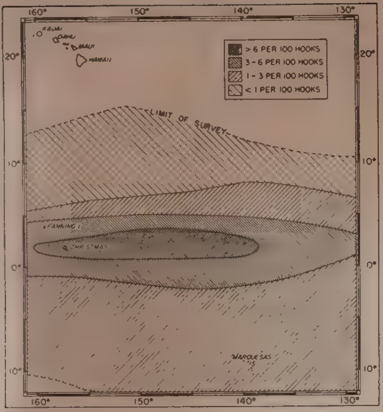

Figure 1—Abundance of yellowfin tuna in the central Pacific.

As is well known to many of our readers, the Pacific Oceanic Fishery Investigations (POFI) was established in 1949 to evaluate the aquatic resources of the central and western Pacific Ocean. As envisioned, the program involved much more than simply conducting superficial surveys of the abundance of fishes; rather, the plan was to study first the oceanic circulation of the area in order to locate regions of the ocean that were favorable to the existence of concentrations of commercially valuable species.

For several reasons, the first area selected for study was the equatorial region south of Hawaii. In this area, available oceanographic information suggested there was upwelling, or enrichment, of the surface waters at and near the Equator. Our initial research confirmed this and, further, indicated that this enriched water moved northward to about 5° N. latitude. It was reasoned that in this general zone between the Equator and 5° N. latitude there would be unusually favorable circumstances for the existence of tuna.

Initial attempts to survey the tuna met with rather sporadic success until the Japanese longline was used. This gear, fished systematically from north to south across the equatorial zone, revealed that there was an unusually dense concentration of large, deep-swimming yellowfin occupying the area shown on the chart (Figure 1).

It is possible that this band of yellowfin could have been located more quickly by eliminating the study of the environment, but this seems doubtful in view of the immense area of the equatorial Pacific and, furthermore, there would have been no way of knowing whether the band of tuna was a permanent concentration or merely a transient population. In contrast, our integrated studies of the circulation, the productivity, the food supply, and the tuna give us confidence that the resource is a reasonably stable feature of the ocean.

The first attempts to fish commercially in the equatorial area followed the extensive exploratory work by POFI which showed that the resource was indeed present during most periods of the year and that the catch rates, on the average, were high enough to support American fishermen. Another factor in the timing of the initial exploitation in early 1954 was the very favorable status of the tuna market insofar as American fishermen were concerned.

The first attempt to fish in the area was made by two vessels from the west coast, the *North American* and the *Alrita.* These vessels, using American crewmen, were rigged for longline fishing and together fished an aggregate of about 120 days in the general vicinity of Christmas Island during the period February to April, 1954. They were aided by POFI in this initial venture to new fishing grounds to the extent of partly defraying the cost of tooling up for a new type of fishing.

Considering the numerous handicaps, such as using new gear and fishing in strange grounds, these vessels did remarkably well. Their catch rates (shown in Figure 2) were high enough to be profitable but not high

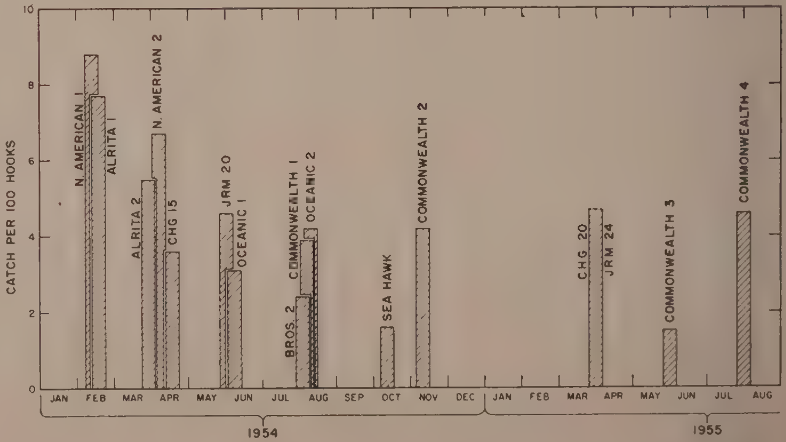

Figure 2—Catch rates in the vicinity of Christmas Island. The first commercial expeditions appear on the extreme left.

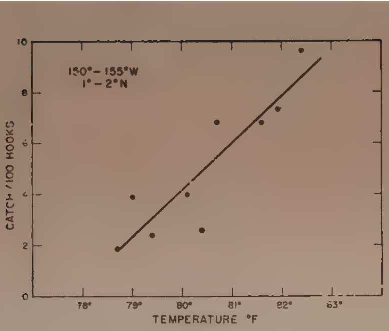

Figure 3—Yellowfin catch and temperature of the surface layer of water (1950-1953).

enough to make the venture spectacularly successful.

Following this initial attempt, there were several other less pretentious ventures into the Line Islands fishery. As shown in the figure opposite, these were not as successful as the earliest trials. Reasons for this lie partly in the use of new and relatively untried gear, but also it is very evident that there was a real decline in the abundance of yellowfin in the Line Islands area during the latter half of 1954, a decline which persisted into 1955.

At first glance it might appear that these experiences are simply a repetition of the frequently observed pattern of good fishing when a new ground is first utilized, followed by a subsequent decline. This argument does not seem reasonable. The area involved is vast and the number of fish taken by the first commercial ventures must surely have been insignificant in comparison to the total population.

In addition, we now have evidence at hand to show that the decline in tuna abundance after 1954 was related to a change in the environment in the Line Islands area. In fact, the poorer fishing might have been predicted had our knowledge then been up to the present level.

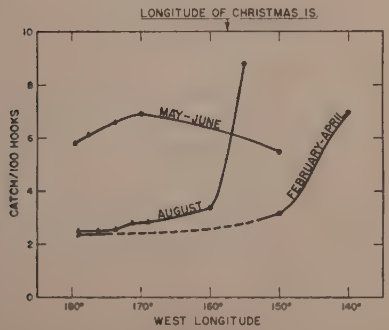

Figure 5—Yellowfin catch 1953, 5°N.-5°S., showing the east-west shifts in abundance.

Briefly, our explanation for this poor fishing follows the logic used in first locating this new fishing ground. We have noted that the newly upwelled water found at the Equator is considerably cooler than the water to the north and south and generally contains few fish. As the water moves northward it "matures," a condition reflected in an increase in temperature, and the following biological succession takes place: the nutrient salts or fertilizers stimulate the growth of minute aquatic plants which, in turn, support an augmented supply of tiny plankton animals and these, in turn, furnish food for tuna forage.

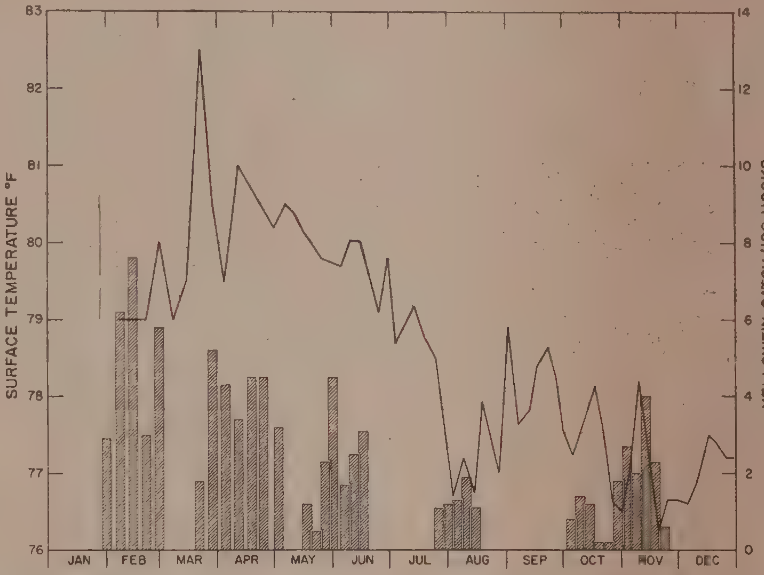

Figure 4—Water temperatures and yellowfin catch rates in the vicinity of Christmas Island, 1954.

The tuna, of course, may be expected to congregate in areas possessing an unusually large supply of food, and to move out of these areas if they suffer a decline in the food supply. According to our theory, a decline in food supply would be indicated by a decrease in temperature.

A recently completed critical examination of experimental fishing results from 1950 to 1953 shows that the success of fishing in the Line Islands area was closely related to the temperature of the water (Figure 3). The very cool water, 78° to 79° F. in temperature, has been recently fertilized and has not yet developed a supply of tuna food.

The warmer water was enriched some time ago, and while it was maturing tuna forage developed, and tuna concentrated in the area. Apparently these variations are the result of variations in the equatorial winds; strong winds drive the water rapidly northward to the sinking of convergence zone and moderate winds allow the tuna forage to develop before the water sinks.

Following this very promising lead, we examined the oceanographic data from our station at Christmas Island and found that much the same story could be derived from this more recent information. The relationship between ocean water temperature and tuna fishing during the 1954 period, when fishing was very concentrated and the catch rates quite reliable, is depicted in Figure 4. It is very clear from this that the decline in fishing success in the vicinity of Christmas Island was paralleled by decline in the water temperature in that area.

Thus it appears that we have a plausible explanation of the poor fishing subsequent to the early part of 1954—an explanation which is related to changes in the equatorial circulation. As in all probability these changes are related in turn to changes in the weather, it is conceivable that more detailed knowledge of the weather may lead eventually to long-range forecasts of fishing success in the equatorial region.

*(Continued on back page)*

# Recent Equatorial Tuna Research

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We have been discussing fishing conditions in the general vicinity of Christmas Island; however, the resource is not tied to Christmas Island or to any other land mass and is, in fact, a fluid resource, moving from place to place in the equatorial system and apparently concentrating where food supplies are greatest. Figure 5 shows our results for 1953, plotted on a latitudinal axis. When fishing is poor at one longitude it may be very much better either to the east or west.

For instance, examine the February-April curve. Fishing was very poor in the vicinity of 180° and rather poor around 150° W., the general longitude near Christmas Island. However, at this same time quite good fishing was found on 140° W., some 600 miles to the east.

Here again, this same set of observations seems susceptible to the temperature analysis we discussed above, for, at the time that the surveys were made, the water was considerably warmer at 140° W. than at 150° W. This is reasonable proof of our theories because, usually, the water is cooler at 140° W. than at 150° W. During the period of this survey the trend was reversed and the warmer water was found to the east and, sure enough, the yellowfin tuna were also found to the east.

Our program in the equatorial area should be continued in the future, for American industry will probably expand in this direction when the economic situation is more favorable than at the present time. We could aid this expansion by two service projects; first, development work to mechanize the longline gear so that it will be a more economical tool in the hands of Americans, and second, electro-sonic fish detection so that we may learn more about the distribution of the tuna and perhaps pinpoint their location. If this could be done it could make it unnecessary to set the longline gear blind.

Over and above this, our "routine" survey program is drawing to a close and our seagoing effort in this area will be restricted to special cruises designed to test the hypotheses, or theories, which have been discussed above. One of the special projects involves following a given body of water as it moves north and west away from the Equator and actually measuring the changes in the nutrient salts, the plankton, the forage fish, and the tuna as the water warms up and passes through the cycle we have described above.

Another program, as yet in the planning stage, is a simultaneous oceanographic survey of the entire equatorial region. This will involve a number of vessels—American, Japanese, and possibly French—and will be designed to cover the equatorial Pacific from the eastern end, where the American fishery exists, to the Philippines. This multiple ship cruise will give invaluable insight into the circulation as a whole and will also serve to tie together the many less extensive surveys that have been conducted in the past, both by POFI and by other agencies operating to the east. *

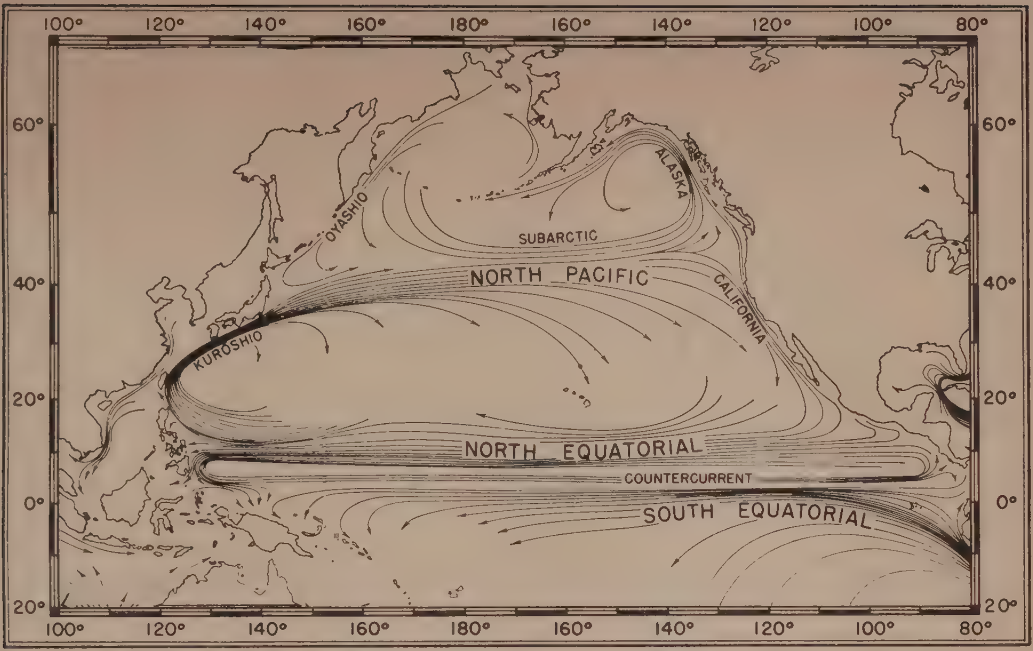

**Figure 1.**—The major surface currents of the North Pacific Ocean.

# The Where and Why of Albacore

### By Albert L. Tester

• In October of 1954 Pacific Oceanic Fishery Investigations received an allotment of funds through the Saltonstall-Kennedy Act for a study of North Pacific Albacore. Since then, we have begun a large-scale effort to explore the ocean to the north of Hawaii and between Hawaii and the mainland in search of potential Albacore fishing grounds. Realizing that this enormous area could not be easily covered by one organization and taking into account the probability that the several apparent populations of Albacore in the North Pacific were closely related, we and our colleagues in the various research agencies of the Pacific states formed a coordinating group known as the Albacore Steering Committee. This committee, which is officially recognized by the Pacific Marine Fisheries Commission, contains representation from the three Pacific states, the Branch of Commercial Fisheries, the Tri-State Commission, and POFI. Its primary purpose is to correlate field work on Albacore so that each of the investigations can make the most efficient use of their available vessels and personnel. In addition, the group meets from time to time to discuss the results of past field work and to lay long-range plans for the future.

In addition to the somewhat formal cooperation described above, the albacore surveys received a spectacular boost this past summer when Fish and Wildlife Service chartered vessels surveying salmon distribution and abundance for the American Section, International North Pacific Fisheries Commission, fished far enough south to show the northern limit of Albacore in mid-ocean, and demonstrated that they could be taken in large numbers in gill nets. It is anticipated that in the future there will be close liaison between Albacore research and the salmon research agencies of the International North Pacific Fisheries Commission.

• *OCEANOGRAPHY*—A schematic version of the surface currents in the North Pacific that are important to the distribution of albacore is shown in the adjoining chart (fig. 1). To the north is the subarctic or Aleutian Current, a sluggish easterly flowing water mass that is comprised largely of cold subarctic water. South of this is the easterly flowing North Pacific Current that is an extension of the warm Kuroshio Current. Proceeding farther south we find the westerly flowing North Equatorial Current, the northern boundary of which lies at approximately 28° N. latitude.

There are two important transition zones in this region. The broad boun-

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dary between the North Equatorial Current and the North Pacific Current is known as the Subtropical Convergence. In the western Pacific the Japanese have a substantial longline fishery extending north of this zone but in the mid-Pacific it has proved unproductive. Lying farther north is the boundary between the North Pacific Current and the Aleutian Current, known as the Polar Front. This zone is characterized by considerably more mixing and turbulence than the Subtropical Convergence and it is in such regions that Albacore occur. In general, in the mid-Pacific their distribution seems to encompass a narrow band of ocean in the vicinity of the Polar Front. This band is displaced to the north in the summer and to the south in the winter.

The hydrographic field studies completed by POFI in mid-ocean should supply definite information on the ecological and physical requirements of the albacore. This may enable research workers and possibly commercial fishermen to predict when and where Albacore will be found. Such knowledge will vastly increase the efficiency of any commercial fishery. In addition, the results of these studies in mid-ocean should provide valuable clues as to why there are variations in the time and place of occurrence of Albacore along the Pacific coast.

● *WINTER DISTRIBUTION* of Albacore in the eastern Pacific insofar as it is known today is succinctly shown in the adjoining figure (fig. 2). Of course there are other occurrences such as sporadic captures off the coast of lower California, but we are here depicting only the occurrence of sizeable numbers of Albacore. It can be readily seen that there is a band extending from 180° eastward to approximately the longitude of the Hawaiian Islands. This band of fish represents an eastward extension of the rather well-defined winter fishing grounds that are regularly exploited by the Japanese to the west of 180°. This zone is of course within fairly easy reach of the Hawaiian Islands and lies rather close to Midway.

It is pertinent to note here that during the last two years Japanese commercial fishermen have experienced very low catch rates in their offshore winter grounds. This appears to be reflected in our results; although we have found fish consistently in this zone during the short period of study, our catches have not been strikingly large. Since the Japanese catch rate during the last two years has been only about one-half of that usually encountered in this area, we fully expect that future surveys will indicate higher concentrations of Albacore to the north of Hawaii.

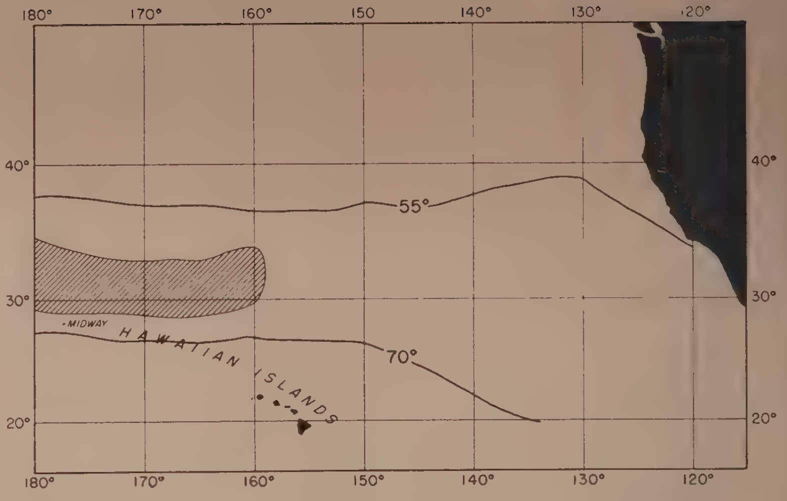

**Figure 2.** — Winter distribution of Albacore in the eastern North Pacific Ocean, shown in area diagonally shaded.

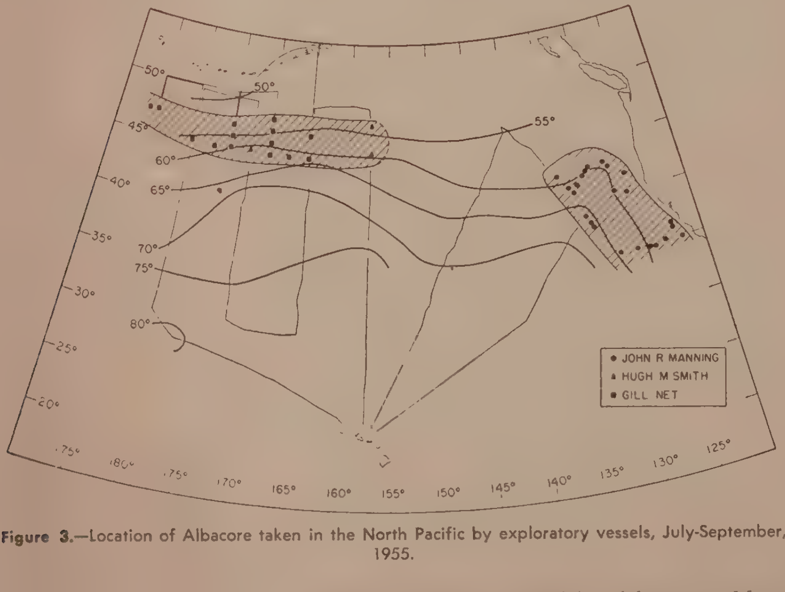

**Figure 3.**—Location of Albacore taken in the North Pacific by exploratory vessels, July-September, 1955.

Whether or not this area is potentially capable of supporting a commercial fishery by Americans remains for the future. All the elements are present; operational bases, fish, and a method of harvesting the fish. Better knowledge of the factors responsible for concentrations of albacore will go a long way towards answering this question. Of course other important factors such as weather, which is stormy during the winter season, and economic trends will doubtless affect development of a fishery.

● *SUMMER DISTRIBUTION*—The summer of 1955 saw a tremendous amount of effort expended in the high seas of the North Pacific. NORPAC, a synoptic oceanographic survey, was mounted. This survey involved Japanese, Canadian, and American vessels and provided for the first time comprehensive coverage of the entire North Pacific, north of 30° N., and should be invaluable in furthering our understanding of the distributions of salmon and tuna in the area. In addition, one POFI vessel and one California vessel were in the field surveying albacore and several vessels were studying the distribution of salmon to the north.

The oceanographic results will not be available for some time but the fishing results have been summarized on the adjoining chart (fig. 3). Here we see a composite picture resulting from the gill net catches of albacore by the salmon gill netters *Paragon* and *Mitkof*,* indicated by squares; the troll catches of the *Hugh M. Smith*, indicated by triangles; and the troll catches of the *John R. Manning*, indicated by solid dots. Also shown on this chart are the tracks of the two POFI ves-

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*Data supplied by courtesy of C. E. Atkinson, Chief, Pacific Salmon Investigations, U. S. Fish and Wildlife Service Seattle, Washington.

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sels and the surface temperature isotherms. These summer surveys show very clearly that there was an extensive body of Albacore in the north central Pacific between 45° and 50° N. Proceeding east, there appears to have been a gap in the occurrence of Albacore until approximately 135° W. longitude. East of this longitude, the *Manning* found Albacore widely distributed off the coast of Oregon and California in a broad band which lay about 300 miles offshore in the north and approached the coast in the south. The southern extremity of the band was, of course, fished by the California commercial fishery.

A little later in the year, that is, during September and early October, POFI fielded another vessel, the *Charles H. Gilbert*, to fish the area to the northeast of Hawaii. This time significant numbers of albacore were taken in the area which had formed a gap in the distribution during August. Fish were caught with longlines, trolling gear and gill net, the last two methods giving the most promising results.

- *TWO ADVANCES*—As a result of the summer and fall work, two important advances have been made. *First* of all we now know that there are large numbers of albacore to the north of Hawaii, constituting a hitherto uncharted body of summer fish. Secondly, and even more exciting, is the fact that unfished Albacore stocks were found some 300 miles off the coast of Oregon and northern California during August.

Again it is not possible to state with certainty the commercial implications of these findings. General surveys such as these are designed to cover as wide an area as possible and usually show little more than the presence or absence of fish. It will take further effort to determine whether or not the populations are abundant enough to support an offshore or mid-ocean fishery in the summer or fall. We hope to make such an effort in the future after we have obtained more precise information on the most probable location of concentrations of fish and, particularly, after we have more clearly delineated the environmental factors that restrict the Albacore to narrow segments of the ocean. The latter is probably the most important objective since it will never be possible to predict when and where to catch Albacore unless we understand the reasons for their occurrence in particular places at particular times. Until this is accomplished fisheries must remain on a hit-or-miss basis.

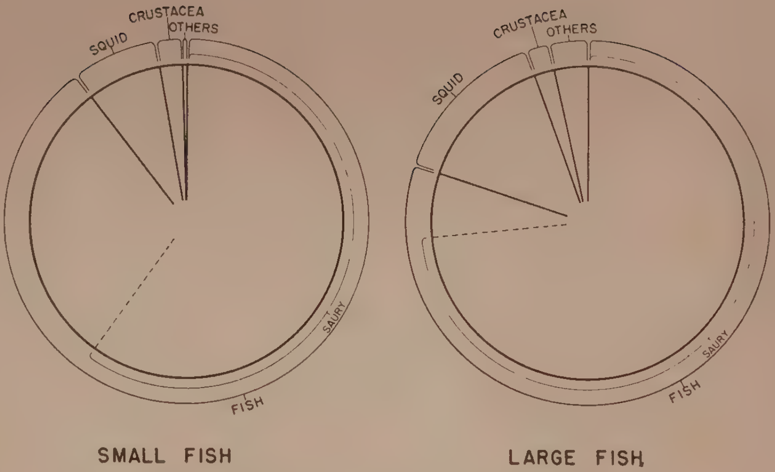

**Figure 4.**—Food of north central Pacific Albacore.

- *FOOD FACTORS*—In addition to studies on the circulation of the water and the distribution of Albacore, a number of supporting programs have been initiated. POFI is conducting an intensive study of plankton organisms in the North Pacific and preliminary results indicate that the occurrence of Albacore is closely related to the occurrence of certain types of readily identifiable plankton organisms. This work should provide another tool in understanding the where and why of Albacore. Other problems must, of course, be explored before we can say that we understand a species of fish and its fishery. Life history work on Albacore is being currently carried out, studies centering around the spawning problem, the growth rate problem, and the food requirements of the Albacore.

An indication of the preliminary results from the food studies is given in the adjacent figure (fig. 4). It is quite evident that the saury, a moderate sized forage fish found widely distributed over the central Pacific, is exceedingly important to the albacore. This suggests that in the process of understanding Albacore we must also understand saury, their distribution, their life history, and the types of water they prefer to live in.

- *IN CONCLUSION* a few general observations may be made. Delineating and predicting Albacore occurrence is a vast undertaking which under present circumstances can be accomplished only by cooperative effort of the several research agencies of the Pacific states and the Federal Government. Observations must be taken over large distances of the North Pacific to obtain an understanding of the offshore distribution, as well as the inshore distribution, both of which are related to each other, and to variation in oceanographic conditions. Coverage less extensive than we are obtaining now will mean that establishing an Albacore fishery based upon an understanding of the conditions which control their distribution will be seriously delayed.

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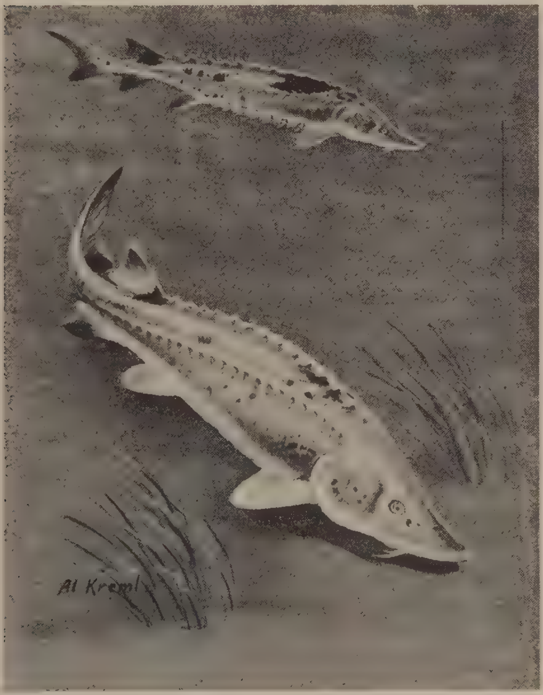
# The Lake Sturgeon

JOHN VAN OOSTEN

Seven species of sturgeon occur in North America: the white and green on the Pacific Coast; the Atlantic and shortnose on the Atlantic Coast; and the shovelnose, lake and pallid in the inland rivers and lakes. All of these varieties have been reduced in numbers, but their existence is not endangered to the same degree in all waters. The United States annual catch on the Pacific Coast still averages 393,000 pounds. Along the Atlantic and Gulf Coasts, however, the yearly take is only about 94,000 pounds, and in the Mississippi River and its tributaries it was 72,000 pounds in 1950. In the Great Lakes the sturgeon has suffered the greatest depletion of any of the once-abundant varieties of the United States. This lake sturgeon has, therefore, been selected to represent one of the most endangered species of its group.



FROM WILDLIFE CONSERVATION STAMP SERIES

The Great Lakes sturgeon, like all other sturgeons, is a primitive fish. It has retained the cartilaginous skeleton of its ancestors. It is easily recognized by its elongated, cylindrical body covered partly by five longitudinal rows of heavy, bony shields (many disappear with age) and by the numerous, inconspicuous, spiny scales. The snout is conical and flattened on the top. Beneath it are four fleshy barbels that are used to locate food on the bottom. Behind them is a protrusible, thick-lipped mouth adapted for sucking up bottom materials from which mollusks, crayfish, insect larvae, worms and other food items are strained. The upper lobe of the tail, like that of a shark, is much longer than the lower. The young are tan or buff-colored with dark blotches on the sides. The adults are darkish above and paler below without spots.

The lake sturgeon is the giant of our inland freshwater fishes. It has been known to reach a length of 8 feet and a weight of 310 pounds. Most of those in the Great Lakes area measure less than 6 feet and weigh below 100 pounds. The species grows slowly at first, but ultimately individuals may reach a large size and an advanced age.

The oldest lake sturgeon on record reached the age of 152 years when it was caught in 1953 in Lake of the Woods. It measured 6 feet, 9 inches and weighed 215 pounds. It is astounding to reflect that this individual was hatched in 1801 and managed to escape the fishermen and its enemies until 1953. In 1943, a fish which was in all probability even older than this, weighing 310 pounds and measuring 7 feet, 11 inches, was taken from Lake Michigan.

The lake sturgeon may reach sexual maturity when about 22 years old (males may attain it earlier). Spawning usually takes place in May and June, either in the larger rivers or in the shallow water of the lakes. A large female may produce 500,000 eggs or more in one season. They hatch in a week at a temperature of 65° Fahrenheit. When about one inch long the young begin to feed on minute crustacea, and at a length of about nine inches they become bottom-feeders.

Although once considered a nuisance in the commercial nets, the Great Lakes sturgeon is now the highest-priced fresh-water fish on the market, bringing the fishermen about $1.25 per pound. Current profits, however, are inconsequential because of the small annual production. The recent average yearly take of United States and Canadian fishermen from all of the Great Lakes amounted to only 40,920 pounds, of which the former produced 14,250 pounds

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DR. JOHN VAN OOSTEN has been associated with fisheries research on the Great Lakes for some 30 years. His work and many publications have brought him recognition as one of the leading fisheries management authorities of the country.

and the latter 26,670 pounds. In earlier years (1879-1890) the combined yield averaged 6,368,300 pounds. Ever since the early nineties this total production gradually declined until the fishery virtually disappeared, the catch having been reduced some 99.4 per cent. This tremendous reduction took place in every one of the Great Lakes.

A telling factor in the decline of the Great Lakes sturgeon was the deliberate efforts of the commercial fishermen to wipe out the species, not only because it had no market value in those very early years, but primarily because the monsters ripped and tore the nets every time they got into them. Later, when a market developed for caviar and smoked sturgeon, the fishermen constructed special gear to take greater quantities of these expensive fishes, both mature and, unfortunately, immature individuals. Spawners and the young were not given proper protection until it was too late to be very effective. Even then, illegal fishing was so extensive that protective measures were of little avail. Early and very destructive over-fishing and man-made changes in the sturgeon's environment became important decimating factors. Among the latter pollution, dredging and damming of those rivers used for spawning were the most serious.

No one can say with assurance that the sturgeon populations of the Great Lakes can be brought back to normal, even if complete protection is provided by law. It is a well-known biological fact that when a population is reduced beyond a certain threshold it is virtually impossible to restore. The lake sturgeon seems to have reached this point, at least in some of the Great Lakes. In Lake Michigan, for example, the species was given complete protection beginning in 1929. In 1951, the State of Michigan again permitted the sale of this fish. What effect did the 21-year closure have on the stock? In the three years (1927-29) preceding the closed season, the average annual catch amounted to 2,282 pounds, a reduction of 99.9 per cent from the normal. In 1951-54, the average was still a mere 2,732 pounds. The year-round legal protection of the species for two decades had no apparent effect on current production.

This failure of the sturgeon to increase was not wholly unexpected. The near extirpation of the species prior to 1930, and the fact that it requires some 20 years, more or less, to attain adulthood makes it obvious that a significant increase in population would require more than two decades, even if a big survival of the progeny was obtained every year. However, the normal survival of fishes in nature is very small, and the female sturgeon does not spawn every year.

The restoration of the Great Lakes sturgeon is a difficult if not a hopeless task. Drastic action is required if it is to be brought back. Year-round protection on both sides of the international boundary and heavy stocking of fish beyond the fry stage in suitable waters seems to be the only methods by which the Great Lakes sturgeon can be rescued from complete extermination. Sporadic fish-cultural efforts of the past have not been too successful, and the species has never been reared beyond the fry stage. Research would, therefore, be needed to overcome these obstacles.

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# New directions in fishery research

By LIONEL A. WALFORD

*The Fishes of the Gulf of Maine*, by BIGELOW and SCHROEDER, published two years ago, brings up to date a work that has been an American ichthyological classic for 30 years. It sums up our knowledge about every species of the northwest Atlantic that regularly occurs in the oceanic bight from Nantucket Shoals and Cape Cod eastward to about 65° W. longitude and including the Bay of Fundy. When read in comparison with the 1924 edition, it measures the progress which biologists have been making in their studies of fishes in this area. In bringing together the work of three generations, it marks where we are now, and serves as a basis on which to consider the question of where to go from here. In the following paragraphs, I propose to examine some facets of this question from the viewpoint of fishery research.

In general, the ichthyological fauna of the North Atlantic is now fairly well known; at least the species which are of actual or potential commercial interest are well known. This does not mean that taxonomy is finished in the Gulf of Maine (or anywhere else for that matter) leaving only the routine job of keeping the bottles on the museum shelves properly labelled and filled with alcohol. What has been done so far is to lay the foundation on which the taxonomy of the future must be built; for there remains the enormously important and difficult work of clarifying conceptions of the sub-populational structure of the species. This is not of " mere academic interest ", as people who are unsympathetic to this line of research sometimes scornfully assert. Fishery investigators have everywhere shown it to be a fundamental necessity; for sub-populations often differ enough from each other in many ways—in rates of growth and mortality, in fecundity, in migratory habits, and in various other biological characteristics, that these segments of species must be studied, fished and regulated as distinct entities.

No matter how distinct they may be genetically, however, they are exceedingly difficult to define, for body proportions and meristic counts vary widely within sub-populations; they overlap broadly between populations; and they are affected by the continually varying conditions of the environment, particularly temperature. Because of the consequent limitations in the usefulness of anatomical features, other means of identifying sub-populations are being sought in the fields of biochemistry, serology, parasitology, and experimental marking. Whatever methods be used for analysing sub-populations, it is necessary to systematically sample large quantities of fish throughout the range of each species under study. In the northwest Atlantic, this laborious and costly work has hardly begun. So far, it has necessarily been concentrated on only the species of greatest commercial importance, namely haddock, cod and red-fish; and even these few species have been studied intensely only over a part of their total distributions. Thus, the study of sub-populations of these must continue, while that of all the other species still lies ahead.

*The Fishes of the Gulf of Maine* is full of information that is best classed as Natural History. There is much more of this than could be assembled for other parts of North

America, since history is longer and the facilities for keeping records go back further there; universities and museums are more ancient, great fisheries and fishery researches have been longer established, and there has been more opportunity to observe the habits of fishes and to record the vagaries of fisheries there than in other sections of the country. Thus over the course of over 300 years there has accumulated a great store of knowledge about western north Atlantic fishes—about the characteristics of their environment, their seasons and places of spawning, the structure of their eggs and larvae, their food habits, routes of migrations, anomalies of occurrences, and many other matters relating to normal patterns of life history and to the deviations therefrom.

Natural History has been unappreciated for a long time, and is now at last again coming into its own, though perhaps under a different label. During the 1920's and 1930's the extraordinary growth of new fisheries such as for haddock in New England, for sardines in California, and for shrimp in the Gulf of Mexico, aroused concern for the future productivity of those natural resources that were being subjected to unprecedented exploitation. It was logical that these fisheries should be regulated as promptly as possible in order to safeguard them from extermination, and that to be effective, the regulations must be based on scientific information. It transpired from preliminary inquiries that a special kind of information was required which in fact did not exist. Although there was a large store of facts, none of it was suitable for calculating estimates of the sizes and ages at most profitable capture. Under the impetus of the demands of the time, therefore, biologists whose interests might otherwise have been natural history took up the study of fishery biology. At first they were preoccupied with determining the effect of fishing on the abundance and productivity of populations of fish. For this they paid the bulk of their attention to founding useful systems of commercial statistics and to the analysis of the quantities, sizes and ages of fish caught in relation to the amount of effort required in the catching. They tended to limit biological studies to those features of life history required for scientific regulation, i.e. rates of growth, death and replacement.

Fishery research might have remained so for a much longer time had it been proved unequivocally that fishing alone causes depletion, which can always be corrected simply by controlling the catch. However, one of the principal results of studies over the past 30 years has been to bring out that, although fishing certainly does affect the abundance of fishes, so also do events which happen in the ever changing environment. Long period trends in climate and hydrography, for example, affect distribution and abundance of all species, whether they are fished or not; and they affect different species in different ways. Thus there is continuous change in the numerical interrelations among the many species inhabiting an environment, perhaps with some kind of pattern of oscillations which has yet to be deciphered.

Fishery researchers had first thought to speed their progress towards practical results by limiting the scope of their studies to the species of greatest importance and to the problems of greatest urgency. Every study about those species, however, has made it more evident that they can not be understood out of context from the intricate system of their biological environment composed by their predators (including man), their competitors and their prey. So it is that fishery researchers have been finding it necessary to rediscover natural history (now called ecology), and to enlarge the scope of their programmes in order to establish a proper balance between studies of fisheries and of fishes. The average fishery programme of today is neither a one-man nor a one-agency enterprise. It generally involves the collaboration of several institutions, sometimes of several nations, and the participation of many scientists having among them a wide variety of disciplines and talents.

Most fishery investigations begin with the tacit hope that a solution to what had looked like a clear-cut problem will be reached, and the work concluded in five or six years. Of course this never happens. Research about the effect of varying exploitation upon irregularly oscillating populations of fishery organisms can not be concluded for all time any more than can research about any other constantly changing natural phenomena. In this connection, it might be helpful in our thinking to alter our concept of the dimensions of time from years to generations. The time required for a brood of fishes to reproduce itself (i.e. a generation) varies from one species to another, but commonly runs about three to eight years. To formulate dependable principles about fishes, it is necessary to follow many generations. Most fishery problems also involve human affairs. Those that concern us now will probably be much more acute a generation hence (i.e. 30 years from now) when people will be more numerous and their food needs consequently greater.

Thus the new directions in fishery research are towards greater comprehensiveness —the whole of species with all their sub-populations; the whole of environments with all their component species. This takes up again the tradition established far back in the past, which is behind books like *Fishes of the Gulf of Maine*, and promises to carry it forward to further enrich human knowledge about the sea.

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## 5

# Hematology of Healthy and Virus-diseased Sockeye Salmon, *Onchorhynchus nerka*[1]

Margaret E. Watson,[2] Ray W. Guenther[3] & Rodney D. Royce[3]

(Plates I-III; Text-figures 1-4)

### INTRODUCTION

Epizootiological investigations have been routine in this laboratory[4] ever since a virus disease began depleting the Federal hatchery stocks of fingerling sockeye salmon in 1951 (Rucker *et al.*, 1953; Watson, 1954; Watson, Guenther & Rucker, 1954). Only cursory observation of stained blood films had been made from diseased fish. More detailed hematological studies were instituted by a short term budget allotment to determine the characteristic blood picture of the virus disease. It was also of interest to find a rapid diagnosis of the disease from either live or stained blood films. In addition, determination of hematocrits was undertaken.

### Literature Review

Hematology of teleosts is still a pioneering field of research. Reports on morphology of normal fish blood cells are not scarce but are contradictory. The best reviews of this subject are found in the doctoral dissertations of Yokoyama (1947) and Katz (1949). Dombrowski (1953) has described the normal blood picture of 15 species of fresh water fish.

The very scant literature on hematological characteristics of diseased fish is described by Katz (1950). The hematology of carp afflicted with stomach dropsy has been discussed by Lyman & Shpolanskaia (1949), Schäperclaus (1953) and Dombrowski (1954). Glucksman & Gordon (1953) have studied the hematology of normal and melanomatous fish.

No reports have been found on the observation of blood cells of fish with a phase-contrast microscope although exploratory and also significant hematological studies of higher vertebrates have been undertaken with this equipment (Bennett *et al.*, 1951, p. 213).

Values for percent. hematocrit (the volume of packed red blood cells) have been found for 10 species of fresh water fish and 4 species of marine fish (Young, 1949; Aserinsky, 1954; Lepkovsky, 1930; Hall, Gray & Lepkovsky, 1924; Wilson & Adolph, 1917; Vars, 1934; Field, Elvehjem & Juday, 1943; Benditt, Morrison & Irving, 1941). The average hematocrit values for fresh water fish varied from 24.8 to 46.1 percent.; for marine fish from 13.5 to 29.5 percent. Methods used for obtaining blood were cardiac or bulbous puncture, severance of tail and pricking of caudal artery above the posterior margin of the anal fin.

It is believed that this study will report the first account of a micro-hematocrit method for small fish (30-60 mm. in fork length). These fish could deliver only one or two drops of blood after their tails had been severed.

### Materials and Methods

The sockeye salmon fingerlings used in this study were reared at the Federal hatchery in Leavenworth, Washington, 130 miles from Seattle, Washington, and were trucked to the laboratory for use in experimental infections. In Seattle, 200 of these fish were kept in running spring water in a baked enamel trough, 4′×1′×6″, held at 50-54° F. The water level averaged 5 ½″. Sockeye fingerlings which contracted the virus disease by natural infection were kept at the Leavenworth hatchery in a trough which contained 13,000 fish at the onset of the disease. These diseased fish were brought to Seattle in lots of 400 for three successive weekends. At Seattle hematological studies were made on fish naturally infected with the virus disease and on fish which had earlier been experimentally exposed to the virus.

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## Experimental Procedures for the Study of the Virus Disease

*Preparation of virus material for experimental infection of fish.*—The original source of the virus for this research was virus-diseased fish naturally infected in hatcheries in the state of Washington. This virus material was passed through at least four serial transfers prior to the making of a frozen stock suspension. The suspension consisted of a frozen (−40° C.) emulsion of infected fish which had been diluted 1:3 with tap water and sealed in glass vials. After six months, this culture was again transmitted through 13 serial transfers before use in the experiment. For this study, fish from the 13th serial transfer were blended in an "Osterizer" for 30 seconds and centrifuged for 3 minutes at approximately 2,000 rpm. The supernatant was then filtered through a 7 lb. Mandler filter. The filtrate was diluted with sterile tap water to a concentration of 1:100. For the artificial infection, 200 fish were suspended for 15 minutes in a gallon of Mandler filtered suspension diluted 1:100. Control fish were given the same treatment as the infected fish except for the exposure to the virus agent.

*Protocol.*—Antecedent to the experiments recorded in this study, four trial infection experiments were carried out in order that hematological characteristics of the virus disease could be determined. Following these, three lots of 200 fish each were used. One lot comprised the control or uninfected fish. On May 11, 1954, the two remaining lots were suspended in 1:100 dilution of infected material for 15 minutes. Beginning on May 12 and daily thereafter through May 20, then again on May 23 and June 7, the following techniques were practised. An average of 6 infected fish and 6 control fish were used daily, and for each a Wright-stained and a peroxidase-stained blood film was prepared. One live film was studied with a phase-contrast microscope, either unstained or supra-vitally stained with brilliant cresyl blue, and one hematocrit was made. Lack of blood or time sometimes prevented observation of a live film on all 12 fish. Blood samples from 73 infected and 61 control fish were analyzed during the experiment.

On May 15, naturally infected fish were brought into the Seattle laboratory from the virus-infected trough at the Leavenworth hatchery. The protocol described above for the experimentally infected fish was followed with these naturally infected fish on May 16, 21, 22, 23, 29, 30, June 1 and 9. Controls were likewise employed with these fish. Sixty-four infected and 28 control fish were studied.

*Preparation of equipment and fish for hemic studies.*—Microscope slides and coverslips were soaked in concentrated nitric acid for a minimum of 4 hours, then washed in running tap water for 4 or more hours, then rinsed in distilled water and finally stored in 95 percent. ethyl alcohol. Slides and coverslips were dried with clean gauze just prior to use.

Fork length, head length, general external appearance and activity were recorded for each fish. The fish was then placed between blotters or covered with gauze in such a way that only the portion of the body immediately posterior to the anus was exposed. This exposed portion was dipped into 95 percent. ethyl alcohol, dried and wiped quickly with gauze. The tail was then severed from the body with scissors by a quick firm snip in the region immediately posterior to the anal and adipose fins. The drop of blood which oozed from the severed caudal vein and artery was touched to a capillary hematocrit tube. After sufficient blood had entered the tube, the remainder of the drop of blood on the fish was first touched to one coverslip from which a stained film would be made and then touched to a second coverslip from which a live wet film or a supra-vitally stained film would be prepared. For these small fish, cover slips used were 15 mm. square, No. 1 thickness.

*Stained blood films.*—All blood films were routinely prepared by the standard coverslip technique (Wintrobe, 1949). One coverslip of the pair used was stained with Wright's stain and the other with peroxidase stain (Hughes, 1953). The freshly prepared and dried films were not fixed in methyl alcohol prior to staining. The peroxidase stain was made with absolute methyl alcohol instead of 95 percent. alcohol, hence fixation was unnecessary.

*Live unstained and supra-vitally stained blood films.*—A freshly cleaned slide was lowered carefully toward a drop of blood on a coverslip until the blood touched the slide. The slide was then upturned and the edges of the coverslip ringed with petroleum jelly. For supra-vital staining, a freshly cleaned coverslip was touched by a very small drop of brilliant cresyl blue (0.1 percent. in absolute ethyl alcohol, freshly filtered). A second clean coverslip was placed over the slip containing the stain, and the two were pulled apart immediately. As soon as the stain on each coverslip dried (a few seconds), the stained coverslip was ready to receive a drop of blood.

*Micro-hematocrits.*—A self-filling capillary tube[5] with a capacity of 5 or 10 lambda or the narrow stem of a red blood cell diluting pipette was used as a hematocrit tube. After a tube was filled with blood, the length of the column of blood was measured with a micrometer calipers which was read to tenths of a millimeter. The end of the tube at which the blood had been introduced was closed with sealing wax. The hematocrit tube was inserted into a piece of thick-walled rubber tubing before it was placed in a centrifuge, thus cushioning it from damage by the sides of the metal centrifuge tube holder.

All samples were centrifuged in a clinical model centrifuge at 3,000 rpm. for 35 minutes, and micrometer measurements of red blood cell volume and total volume were recorded within half an hour after centrifuging. If the volume of blood recorded before centrifuging varied more than 1 mm. from the volume measurement of total blood after centrifuging, the sample was discarded.

The use of an anticoagulant was necessary. The hematocrit tubes were rinsed in a solution of heparin (0.25 mg./cc. of sterile distilled water) and blown dry with a jet of air, rinsed a second time with a fresh drop of heparin solution and again blown dry.

Hematocrit tubes were cleaned first by ejecting the wax plug with a fine wire and then by rinsing several times in normal saline and 95 percent. ethyl alcohol. Occasionally a rinse in concentrated nitric acid was required to clean the tube thoroughly.

*Phase-contrast microscopy photographs.*—An American Optical phase-contrast microscope was used with an American Optical (No. 735) spherical microscope illuminator or a Wilcox Strobolite with or without ground glass interference. A 1.8 mm. medium dark-contrast objective was used. The camera was a 4×5 Speed Graphic adapted for photomicrography.

## MORPHOLOGY OF NORMAL BLOOD CELLS

Time permitted only a few blood counts on normal and diseased fish. Hence, the following account can only be qualitative. Normal, apparently healthy fingerlings provide a very uniform blood film for study. Mature erythrocytes are so numerous that one must examine the blood film carefully to detect the presence of the few lymphocytes and clusters of thrombocytes. Occasionally a neutrophil is seen as the only representative of a granulocyte.

One to 2 percent. of the erythrocytes are orthochromatophil or polychromatophil normoblasts. They can be distinguished from the mature erythrocytes by their chromatin pattern, characteristics of the mitochondria and the size of the nucleus relative to the size of the cytoplasm.

In the nucleus of a mature erythrocyte (Plate I, Fig. 4, left of center), the nuclear membrane is very thin, the chromatin particles are small and form a tightly meshed network. Two examples of an orthochromatophil normoblast seen in Plate III, Fig. 13, show that the nuclear membrane is more distinct than that in a mature erythrocyte and that the chromatin particles are slightly larger and less tightly meshed. In a polychromatophil normoblast (3 cells in Plate III, Fig. 14), the chromatin particles are large and form a coarse-stranded meshwork; the nuclear membrane is definitely thicker than that seen in the mature erythrocytes.

Mitochondria in a mature erythrocyte are few in number (8-12) and appear as tiny dots or short rods. In an orthochromatophil normoblast the number of mitochondria is roughly twice that found in a mature erythrocyte, and the majority of them are short, fat rods, although a few may be long, thin rods. In a polychromatophil normoblast, the majority of the mitochondria are long, very thin rods which sometimes appear tangled or interwined. The cytoplasm of the polychromatophil normoblast is crowded with mitochondria. In a live blood film, stained supra-vitally with brilliant cresyl blue, the mitochondria in all erythrocytes become a light shade of blue when examined under bright-field microscopy.

The cytoplasmic area in a polychromatophil normoblast is less than that in either an orthochromatophil normoblast or a mature erythrocyte. The cytoplasm of a mature erythrocyte appears to cover a large surface area because the nucleus is slightly smaller than that of the polychromatophil or orthochromatophil normoblast. The nucleus in both the mature erythrocyte and in the orthochromatophil normoblast is oval, while that in the polychromatophil normoblast is usually more round than oval.

The size of the 3 erythrocytes just described is variable and is directly related to the proximity of either an impending or recent mitotic division. Cells which have recently divided can be as small as 17 microns on their long axis. Cells near division can be as long as 22 microns. The average dimensions of the majority of mature erythrocytes are 19×11 microns. The average nucleus measures from 7-9×4-6 microns.

A basophil normoblast, rarely seen in the blood films of healthy fish, is labeled *BN* in Plate I, Fig. 4. The nucleus and cytoplasm have almost the same index of refraction, and the nuclear chromatin appears homogenized but does contain indistinct areas of chromatin agglomerations. A thin, but definite, nuclear membrane is present, and occasionally the chromatin forms small thickenings on the inner surface of the nuclear membrane. Mitochondria are long and so thin that they are difficult to discern. Occasionally the mitochondria are arranged like small beads on a string instead of being thin, smooth rods.

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[5] Obtained from Wakefield Industries Corporation, Skokie, Ill.

The leukocytes seen in normal sockeye salmon fingerlings are lymphocytes, thrombocytes (the nucleated homologue of the human platelet) and neutrophils (heterophils). A small active lymphocyte, labeled *L*, is shown in Plate III, Fig. 15, and a small quiescent lymphocyte occupies the center of Plate III, Fig. 16. The active lymphocyte has a round nucleus which occupies almost the entire cell, and that part of the cytoplasm which is visible forms a pseudopod containing short rod-like or small dot-like mitochondria. In Plate III, Fig. 16, the mitochondria point inward toward the indented part of the nucleus. These mitochondria could not be resolved photographically. An example of a large lymphocyte, which is seldom seen in healthy fish, is pictured at the lower right of Plate II, Fig. 9. The mitochondria in this large lymphocyte are larger than those in the small lymphocyte and form either long rods or spindles. In both large and small lymphocytes, the nuclear membrane is etched sharply. The chromatin in the small lymphocyte is coarsely clumped, and the open meshwork between chromatin strands is refractile (Plate III, Fig. 16). The chromatin in the large lymphocyte consists of coarsely meshed strands, but the open meshwork is less refractile than that in the small lymphocyte. Neither the small lymphocytes (5 to 10 microns in diameter) nor the large lymphocytes (18 to 25 microns in diameter) display movement other than a very slow oozing or rearrangement of the cytoplasm.

Small lymphocytes and thrombocytes appear quite similar when a live blood film is examined for the first minute after its preparation. Following the first minute, the thrombocytes bleb or balloon out, thus performing their natural function of clotting. A blebbed thrombocyte occupies the right center of Plate I, Fig. 1. The white circular dot near the upper border of the thrombocyte is seen only in virus-diseased fish. The fine threads of fibrin can be seen radiating from the periphery of the cell. An unblebbed thrombocyte in a fingerling sockeye salmon would resemble the lymphocyte pictured in Plate III, Fig. 16, except that the nucleus of the cell would have only a small crevice instead of a deep indentation or the nucleus would completely fill the cell. Only 3 or 4 tiny round granules may be seen in the cytoplasm of a thrombocyte. As a fingerling sockeye salmon approaches a length of 50 mm., a few mature thrombocytes can be seen. These are similar in appearance to the size and shape of the nucleus in a mature red blood cell. A thin, almost imperceptible, rim of cytoplasm encircles the nucleus. In live or stained films from healthy fish, the thrombocytes are usually clumped in groups of 2, 3 or 4.

The most motile cell in a live blood film is the neutrophil or heterophil. As a rule the nucleus trails while the cytoplasm leads the direction of motion. A nonfilamented neutrophil is pictured at the center right of Plate I, Fig. 3. The white refractile dot in the cytoplasm is only characteristic of neutrophils in fish afflicted with the virus disease. This non-filament neutrophil is considered a metamyelocyte which can be recognized by the appearance of the nucleus in an active cell; the nucleus alternately has a hump (as in Plate I, Fig. 3) or a depression midway along its length, as if it performed an undulating motion. The cytoplasm also contains a few tiny round granules which appear slightly darker than the mitochondria. Metamyelocytes are not common in the blood of healthy fish.

The bi-lobed neutrophil is normally the predominant stage seen in the peripheral blood, although highly polymorphic nuclei with 3, 4 and 5 lobes are also occasionally seen. No promyelocytes or myelocytes could be identified as such in any live films examined. After the peroxidase test, the cytoplasm of all neutrophils, regardless of their maturity, was replete with purplish black granules.

Basophils and eosinophils are easily identifiable with phase-contrast microscopy. The eosinophil is a small round cell (8-12 microns) which is entirely filled with minute refractile granules (1-2 microns). These granules are as luminous as a mass of tiny, very bright, light bulbs. The nucleus was rarely seen. When visible it was thin and flattened at one edge of the cell membrane. An eosinophil has never been seen in motion.

Though eosinophils and basophils are uncommon in healthy fish, from 5 to 10 of each have been seen on a single blood film from starved fish, virus-diseased fish and fish infected with bacterial gill disease. Plate II, Fig. 8, depicts one type of basophil which is the same size and shape as the eosinophil just described. Instead of very refractile granules, the basophils have very dark granules which are encircled by a bright halo when viewed under a phase-contrast microscope. The nucleus of the basophil in Fig. 8 is not visible. All of the granules, which really fill the entire cell, are impossible to focus in the same plane; thus the pictured cell appears to be only partially filled with granules. On the other hand, the majority of the granules filling the cytoplasm are visible in the basophil pictured in Plate II, Fig. 9. This cell is flattened, while the basophil in Fig. 8 is rounded. In a flattened basophil, the nucleus is eccentric and has a coarse network of chromatin which is disposed in thick clumps at points of anastomoses. No instance of a polymorphic nucleus has ever been seen in live or stained films. The basophils in Figs. 8 and 9 showed no signs of movement; however, a third type of basophil illustrated in Plate II, Fig. 7, glided slowly with the cytoplasm trailing the nucleus. This is an immature cell, there being fewer and smaller cytoplasmic granules. The diffuse chromatin reveals a faint network.

The granules in all three types of basophils just described have in common an unusual internal characteristic. Each granule contains 2 or 3 parallel striae which appear darker than the granule itself. In addition these granules are specifically stained a deep bluish-purple by the supra-vital dye, brilliant cresyl blue. No other cellular component in the blood of sockeye salmon is so colored by this stain. When one is searching for the presence of basophils in a supra-vitally stained blood film, they can be easily detected by their purplish color during examination with bright-field microscopy and 100 magnification. Corroborating evidence for the identification of basophils of teleosts by their supra-vitally stained granules may be found in Michels (1938, p. 327).

### MORPHOLOGY OF BLOOD CELLS ASSOCIATED WITH THE VIRUS DISEASE

When viewing either a stained or live blood film from a fish heavily infected with the virus disease, one is immediately impressed with the tremendous amount of cellular debris and unidentifiable particles present in almost every field of observation. From a study of a stained film alone, it would have been impossible to determine the origin of this debris, examples of which are shown in Plate I, Figs. 2, 4 and 5, and Plate II, Figs. 10 and 11.

Besides the cellular debris in the blood, the next most obvious characteristic is the delayed clotting time. Ordinarily, with healthy fish, one must work with utmost speed when preparing live or stained blood films in order to prevent clotting, which occurs in about 15 or 20 seconds. Blood from a virus-diseased fish will remain fluid as long as 3 minutes after exposure to air.

At the first sign of decreased clotting time, the thrombocytes in a Wright-stained blood film have a smudged, unevenly faded blue appearance, instead of the normal, homogeneously dark purple. They may or may not have a tiny, dark brown, round pigment spot in their cytoplasm. This pigment spot is about 1 micron in diameter when it is round, but it also may appear as a short fat rod (2×1 microns). With phase microscopy, this included pigment spot appears as a refractile dot. The thrombocyte in Plate I, Fig. 1, is in its earliest stage of degeneration and possesses an intracytoplasmic refractile dot. Plate I, Fig. 2, shows a totally degenerated thrombocyte with 3 pairs of pigment granules. If a live film is examined with bright-field microscopy, these refractile dots appear dark brown to black. These pigment granules have no affinities for stain in a Wright-stained film but do retain the dark brown color which is seen in a live film under bright-field observation.

A totally degenerated thrombocyte, as seen in Plate I, Fig. 2, with phase-contrast microscopy in a live mount, cannot be recognized as such in a Wright-stained film. One can only see the pigment granules in a stained film with bright-field microscopy; however, if the stained film is examined with a phase-contrast microscope, the outline of the degenerated thrombocyte with its included pigment granules is made visible. Variously diseased salmonoids other than sockeyes have been examined and no live or stained blood film has disclosed degenerated or degenerating thrombocytes with included pigment granules. Pigment granules free in the serum are occasionally seen in healthy fish but their presence has been attributed to leaching of pigment from the incised tissues of the tail region during the preparation of the blood film.

During one phase of the virus disease there exists a true basophilia which reveals the existence of the three types of basophils described earlier and pictured in Plate II, Figs. 7, 8 and 9. The majority of the basophils are of the type seen in Fig. 9. Gradually the large numbers of normal-appearing basophils are replaced by degenerating basophils, an example of which is illustrated by Plate II, Fig. 10. The degenerating basophil usually does not have pigment granules, but in a few instances they have been seen. Fig. 10 indicates the pigment granule as a white circle with a gray center dot. The granules in a degenerated basophil are irregular in shape rather than round and do not take a brilliant bluish-purple stain but rather a faded blue color in a film supra-vitally stained with brilliant cresyl blue. The fine striae seen in the granules of normal basophils no longer exist in the irregularly-shaped granules of the degenerating basophil.

Another characteristic of the blood picture in a virus-diseased fish is clasmatosis—the dissemination or scattering of basophil granules, and to a far lesser degree, the dissemination of eosinophil granules in the blood stream. The extracellular granules of basophils are usually enclosed in bits of cytoplasm and appear singly, paired or as many as four in a group (Plate II, Fig.11). In a Wright-stained film there have been only single, isolated instances when these extracellular granules have been located adjacent to or in the immediate vicinity of mature basophils. It is not believed that the granules represent an artifact produced by the mechanical rupture of the basophil. In a live film, they do not possess the fine striae which exist in the intracellular granules. Also, these extracellular granules take on either no color or only a faded blue color in a blood film supra-vitally stained with brilliant cresyl blue. Macrophages have been observed with faded blue granules recently included in their cytoplasm (Plate I, Fig. 6) Phagocytized eosinophil granules have not been observed.

After the peak of mortalities has passed, engorged and degenerating macrophages outnumber all other leukocytes. The nuclear chromatin forms a diffuse spongy network; the cytoplasm has long thin mitochondria and a few dark round granules (Fig. 6) Macrophages are monstrously large cells—one occupying the space which three normal erythrocytes would fill if placed side by side. Little or no ameboid movement was witnessed in macrophages. As many as 12 pigment granules have been seen in an engorged macrophage. Plate I, Fig. 5, shows a macrophage which had recently ingested a large degenerated lymphocyte. The nucleus of this macrophage is a thin lunate structure pushed toward the periphery of the cell.

The presence of many large lymphocytes is also characteristic of the blood of fish severely infected by the virus (Plate II, Fig. 9, center right). Degeneration in the large lymphocyte is manifested by an included pigment granule or cytoplasmic vacuolization, or both. The vacuoles are not refractile. In a Wright-stained film, a degenerated lymphocyte has a thin rim of pale blue cytoplasm and a gray to grayish-blue nucleus. A large normal lymphocyte has a reddish-purple nucleus.

The large leukocyte seen in Plate II, Fig. 12, is as yet unidentifiable. It has rarely been found in the blood of healthy fish. Its cytoplasm differs from that of all other leukocytes in that it contains only small spherical granules which are dark and do not resemble the mitochondria seen in other leukocytes. These granules are about one-third the size of those found in a basophil. Some overlie the nucleus, which appears as a diffuse mass of chromatin circumscribed by a thin membrane. The cell has a slow creeping motion with the cytoplasm leading the nucleus. No counterpart for this cell in a stained film could be found. Further study on larger numbers of these cells is necessary before a definite identification can be made. At this time it will be referred to as a monocyte.

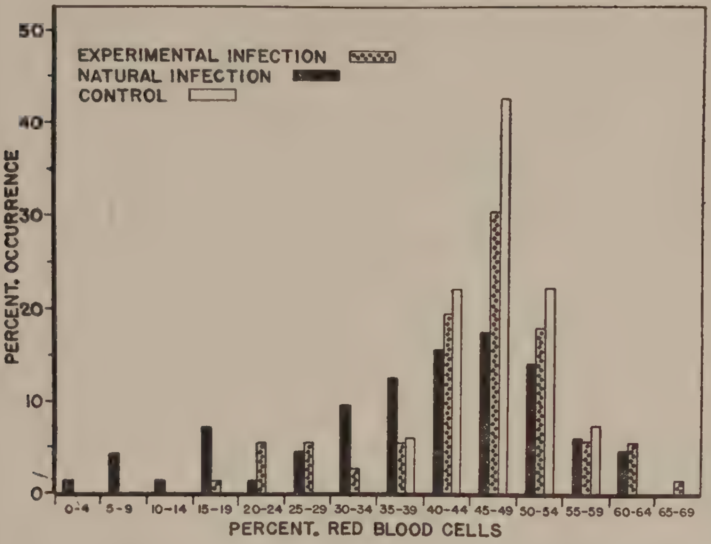
In fish which are recovering from the virus disease there is an extremely high increase in the number of immature erythrocytes (basophil normoblasts particularly). Many of the immature erythrocytes are undergoing mitosis, which is rarely seen in the blood of healthy fish.

### HEMATOCRITS

*Healthy fingerling sockeye salmon.*—The average percent. hematocrit of 68 control fish was 47, representing a range of 35.8 to 57.9 and a variation of 38 percent. (Text-fig. 1). The fish whose hematocrits were in the range of 35 to 39 and 55 to 59 percent. (8 fish) did not present a truly healthy blood picture upon analysis of their Wright-stained films. A variety of abnormalities was represented, neutrophilia and anemia being the most prevalent. They did not show any evidence of the virus disease.

The hematocrits of the control fish used in conjunction with the experimentally virus-infected fish are plotted in the upper part of Text-fig. 2. Beginning the seventh day after artificial infection, the controls also showed evidence of infection not only from the analysis of the live and stained blood films but also from the rise of the average percent. hematocrit. It is not possible to ascertain how the accidental contamination of the control trough occurred. Both the controls and the infected fish were kept in the same hatchery; however, separate equipment was assigned for each trough. The main stock of healthy fish, from which all experimental fish were obtained, was kept in a different hatchery. These stock controls did not acquire the virus disease; hence, accidental contamination was presumed to have occurred in the controls used. A new group of control fish was selected from the stock of healthy controls for obtaining the data listed under the twelfth and twenty-seventh day after infection.

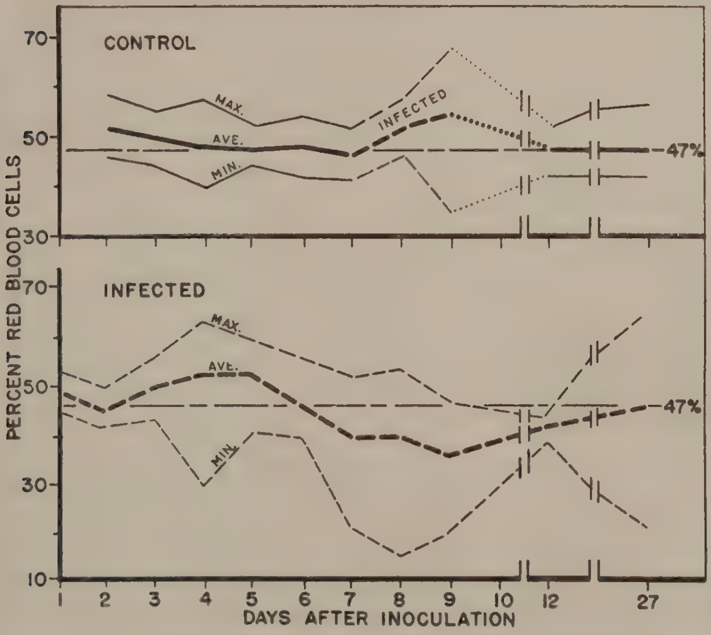
*Fingerlings experimentally infected with the virus.*—The average peak hematocrit was 54.8 (maximum 68) for the accidentally infected controls and 53 (maximum 64) for the experimentally infected fish. For the latter group this peak occurred on the fourth day after infection. From Text-fig. 3 it is possible to correlate the average hematocrit with the percentage of daily mortality. Two days following the peak hematocrit, the maximum number of mortalities oc-



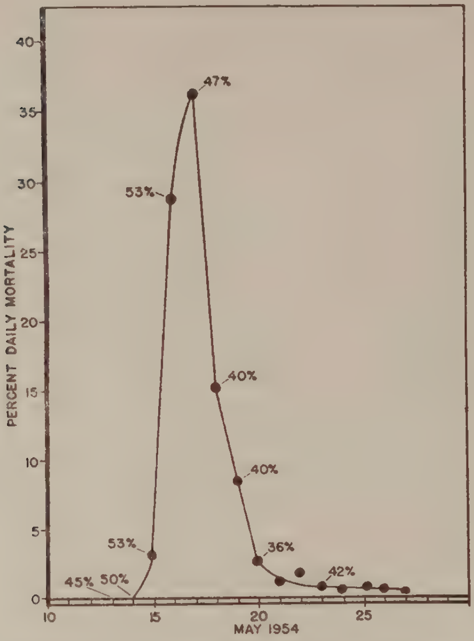
TEXT-FIG. 1. Hematocrit values of fingerling sockeye salmon — control and virus-diseased.

curred—35 percent. of the total fish inoculated with the virus. The explanation for this may be that, because of the shock incurred from the infection, there is a loss of plasma which accounts for the rise of average percent. hematocrit; following the effects of shock, the plasma level and the normal percent. hematocrit (47) are restored in the surviving fish by the sixth day after exposure to the virus.

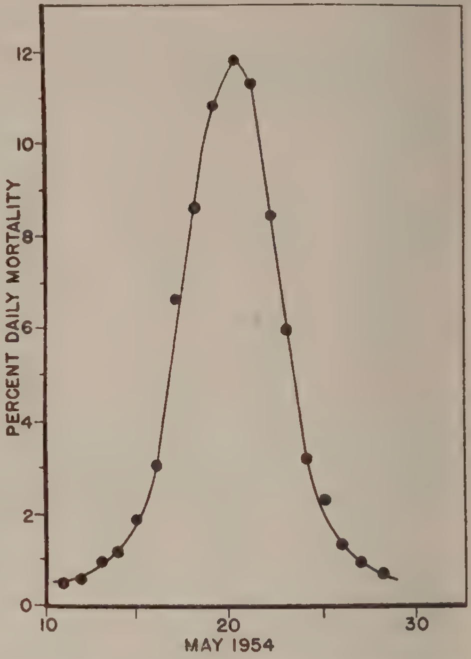
*Fingerlings naturally infected with the virus.*—On May 12, 1954, only 4 fish survived transport to Seattle from the disease-infected trough at the Leavenworth hatchery. It was possible by the examination of live blood films of these fish with a phase-contrast microscope to detect the typical blood cells associated with the virus disease heretofore studied only in experimental infections. The next lots of diseased fish were trans-



TEXT-FIG. 2. Maximum, minimum and average hematocrit values of control and experimentally virus-infected fingerling sockeye salmon.



Text-fig. 3. Percentage of daily mortality of fingerling sockeye salmon experimentally infected with the virus disease, and average hematocrit values of moribund fish delineated on the mortality curve.



Text-fig. 4. Percentage of daily mortality for the duration of the virus infection in a trough containing 13,000 fingerling sockeye salmon naturally infected at the hatchery in Leavenworth, Kansas.

ported to Seattle on May 16, 21 and 28. Since an insufficient number of samples was studied from the onset of infection up to the day of peak mortalities (May 11-May 20), complete hematological data for the entire course of the disease could not be tabulated as was done for the experimentally infected fish (Text-fig. 2).

From May 1 to May 10 the daily mortalities in the diseased trough at Leavenworth ranged from 8 to 24 fish a day. On May 11 there were 65 dead and on May 12 there were 89. A cumulative mortality of 50 percent (6,500 fish) was recorded on May 20, on which day also occurred the peak mortality (1,620 fish) (Text-fig. 4). By May 28 the cumulative mortality had reached 80 percent. These mortalities were comparable both in total magnitude and daily incidence to those which had occurred during the virus epidemic at this same hatchery the preceding year (Watson, Guenther & Rucker, 1954).

The highest hematocrit recorded for the naturally infected fish was 64 percent. on May 21. The lowest hematocrits were 16 percent. for the experimentally infected fish and 1 percent. for the naturally infected fish. Beginning May 22 and continuing through June 7, hematocrits below 35 percent. were recorded for 30 percent. of the fish examined. By contrast, only 15 percent. of the experimentally infected fish registered hematocrits below 35 percent. Infected fish in both groups registering hematocrits below 35 percent. displayed erythema at one or more of the following areas: base of any or all of the fins, at the anus and the isthmus. These low hematocrit values were probably caused not only by the erythema but also by the necrosis seen in hemapoietic areas of kidney tissue sections.[6]

*Microscopic findings related to the hematocritis.*—The hematological schedule on page 35 was compiled, presupposing one single fish could have been bled every day from the onset of exposure to the virus infection. These data are based on the results obtained from both experimentally and naturally infected fish.

## Discussion

This study has demonstrated that phase microscopy provides more useful visibility of live blood cells than does bright-field microscopy of stained blood cells. During the course of the virus disease, certain blood cells were difficult to differentiate with certainty by their staining characteristics because they were undergoing de-

| Days after Infection | Percent. Hematocrit | Microscopic Blood Findings |
|---|---|---|
| 1 | 45-50 | No change from the normal. |
| 2 | 45-50 | A few thrombocytes possess one pigment spot; increase in number of metamyelocyte neutrophils. |
| 3 | 50-55 | |
| 4 | 55-65 | Majority of thrombocytes and neutrophils contain one to four pigment spots; delayed clotting time; a few basophils; first mortalities occur. |
| 5 | 65-55 | Degenerated thrombocytes and neutrophils; a large number of basophils (10-12 on each blood film); a few large lymphoctyes, eosinophils and macrophages; extracellular granules of basophils; by the end of the sixth day maximum daily mortalities occur. |
| 6 | 55-45 | |
| 7 | 45-35 | Large number of macrophages; pigmented degenerated thrombocytes, neutrophils; basophils and large lymphocytes; extracellular granules of basophils. |
| 8 | 35-10 | Immature erythrocytes outnumber mature erythrocytes; mitotic division seen in many of immature erythrocytes; macrophages; many unidentifiable particles of degenerated cells; clotting time still prolonged. |
| 9 | 10-30 | |
| 12 | 30-40 | Polychromatophil and orthochromatophil normoblasts in minority, no basophil normoblasts; a few normal neutrophils and thrombocytes. |
| 27 | 40-50 | Clotting time normal; blood picture normal; first evidence of scoliosis in recovered fish. |

generation or were completely degenerated. The possible vagaries and variations in stains and staining techniques make it difficult to reproduce results. The characteristic striae seen on each basophil granule were not seen in a stained blood film, nor were they reproducible photographically. Thus, they could only be observed by phase microscopy.

Basophilia has not been recorded in published reports as a diagnostic feature of any other fish disease. The hematology of stomach dropsy in carp has been described in two separate studies. Dombrowski (1954) reported a decrease in lymphocytes and an increase in leukocytes, followed by degeneration of the leukocytes (a term which Dombrowski used without qualification). Lyman & Shpolanskaia (1949) also reported a decrease in lymphocytes, but were specific in naming the types of increased leukocytes, although they did not describe them. At the severest stage of infection, monocytes increased from the normal 5.7 to 38 percent., neutrophils from 0.2 to 7.0 percent., polymorphonuclear cells from 2.1 to 11.2 percent. The type of cells included in the term *polymorphonuclear cells* was not stated. Eosinophils were not found in the peripheral blood until the third year; the term *basophil* was not mentioned.

Besides basophilia, other notable characteristics of the virus disease are prolonged clotting time, the existence of pigment particles in the degenerating thrombocytes and neutrophils, and extracellular granules of the basophils. None of these characteristics has been delineated for any other fish disease described in the literature.

Beside microscopic blood findings, a sharp rise in percent. hematocrit values indicates the onset of the virus disease. However, future studies may prove that high hematocrit values are also corollary to other infections in fish. In two other instances, hematocrit values of fish have risen above the normal. Vars (1934) found that the toxicity of ammonium salts resulted in raising the mean percent. hematocrit of carp from 32 to 39. Menhaden have been exposed for various intervals of time to an asphyxiating environment by Hall, Gray & Lepkovsky (1924). The increase in hematocrit values from the normal (29.5) to 68.9 percent. was roughly proportional to the length of time of asphyxiation. These high hematocrit values of asphyxiated menhaden were attributed to the effect of the release of water from the blood to the tissues.

For the naturally infected fish, the successive number of days in which mortalities occurred ran twice as long (17 days) as it did for the experimentally infected fish (8 days). The longer duration of the disease could be the result of (1) residence in water cooler by 10° F. than that in experimentally infected fish troughs; (2) spread of the infection rather than simultaneous exposure of all fish; (3) difference in route of infection.

## ACKNOWLEDGEMENTS

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[6] Unpublished data.

ty of Washington, for his guidance and criticism; Mr. E. F. Marten, Supervisor of Still Photography Production Unit, University of Washington, for his expert assistance with the photomicrographs; and Mr. Vladimir Lipski for his Russian translations.

## Summary

Observations of cell types found in the peripheral blood of healthy and virus-diseased sockeye salmon fingerlings (*Onchorhynchus nerka*) are made with a phase-contrast microscope and reproduced photographically. Cells described include erythrocytes, thrombocytes, lymphocytes, neutrophils, eosinophils, basophils and macrophages.

Two diagnostic characteristics of the virus-diseased fish are: (1) before mortalities occur, degeneration in leukocytes and thrombocytes is made evident by the presence of an intracytoplasmic, highly refractile body which appears as a dark brown pigment granule when viewed with bright-field microscopy; (2) during the severest stage of the infection, extracellular granules of basophils appear, as well as totally degenerated leukocytes and thrombocytes which are only visible with phase-contrast microscopy.

The average percent. hematocrit of 68 control fingerlings (30-60 mm. in fork length) was 47. Hematocrit values were determined for fish which had been experimentally and naturally infected with the virus disease. At four days after experimental infection, the percent. hematocrit rose to an average of 53 (maximum 64); at 6 days, peak mortalities occurred and moribund fish registered a normal percent. hematocrit. Hematocrit values fell to a low of 16 percent. by the eighth day after exposure to the virus and then gradually rose to normal by the 27th day. Associated with below normal hematocrit values were erythematous areas at the base of the fins, at the anus or the isthmus. Microscopic blood findings and corresponding hematocrit values were related. Microhematocrit tubes used were capillary pipettes (5-10 lambda).

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## EXPLANATION OF THE PLATES

All figures are photomicrographs of blood cells found in live wet films of peripheral blood which was viewed with a phase-contrast microscope.

### PLATE I

FIG. 1. Cell in upper right is a blebbed thrombocyte. The colorless circular area near the upper margin of this cell is a refractile body which is a pigment granule whose presence is the first evidence of cellular degeneration and denotes a virus infection.

FIG. 2. In the upper half of the field is a totally degenerated thrombocyte containing 3 pairs of pigment granules which appear as refractile bodies, each outlined by a thin dark line. In the lower half of the field is a senile erythrocyte containing a pycnotic nucleus.

FIG. 3. A non-filamented neutrophil (metamyelocyte) occupies the center and right portions of the field. Its cytoplasm contains a refractile body common in a virus infection.

FIG. 4. A normal mature erythrocyte is located left of center. Basophil normoblast is labeled *BN*. Center of the field is occupied by a totally degenerated neutrophil found in a virus-diseased fish.

FIG. 5. Macrophage which has recently ingested a large degenerated lymphocyte is characteristic of virus-diseased blood. Nucleus of macrophage is compressed at the upper right margin of the cell.

FIG. 6. Macrophage containing phagocytized granules of basophils which are numerous in a virus-diseased fish.

### PLATE II

Cells characteristic of blood from a virus-diseased fish.

FIG. 7. Immature basophil with only a few small, dark granules and a large nucleus.

FIG. 8. Small, round basophil filled with dark granules; nucleus is not visible.

FIG. 9. Large lymphocyte at lower right, above and to the left of which is a basophil.

FIG. 10. Degenerating basophil.

FIG. 11. Three groups of extracellular granules of basophils: upper left is a pair of granules, extreme lower right is a single granule, above which is a pair of granules unequal in size.

FIG. 12. Large leukocyte not positively identifiable but called a monocyte.

### PLATE III

FIG. 13. Polychromatophil normoblast between two orthochromatophil normoblasts from a virus-diseased fish.

FIG. 14. Three polychromatophil normoblasts from virus-infected blood.

FIG. 15. *L* is an active small lymphocyte with a pseudopod. A polychromatophil normoblast is to the right of the lymphocyte. All other cells are mature erythrocytes (healthy fish).

FIG. 16. Small quiescent lymphocyte (healthy fish).

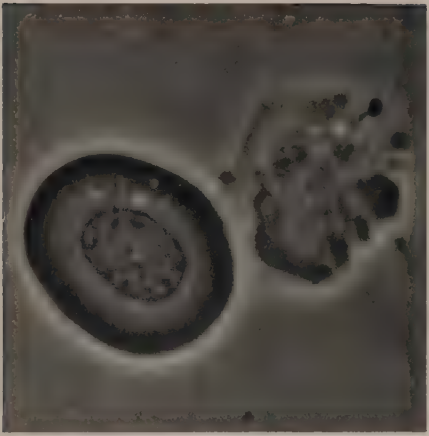

FIG. 1

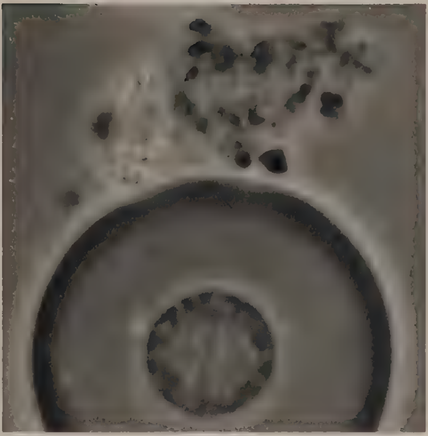

FIG. 2

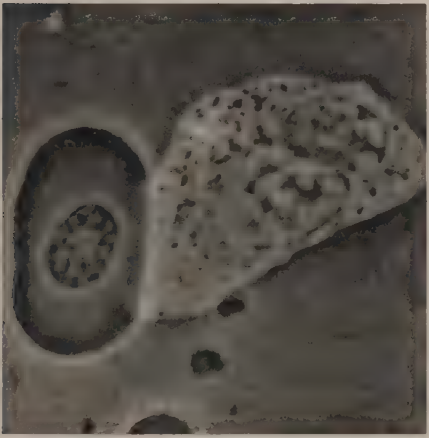

FIG. 3

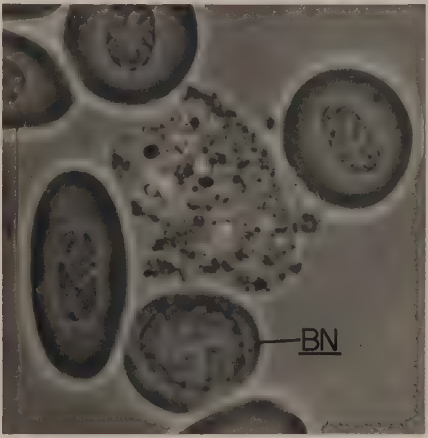

FIG. 4

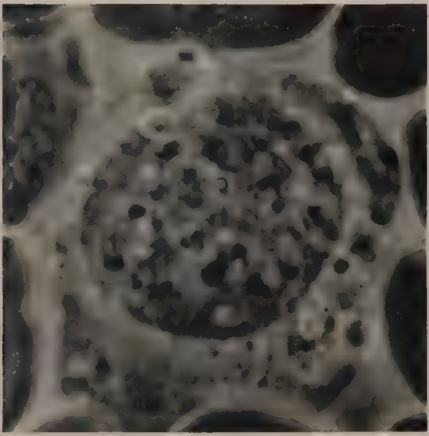

FIG. 5

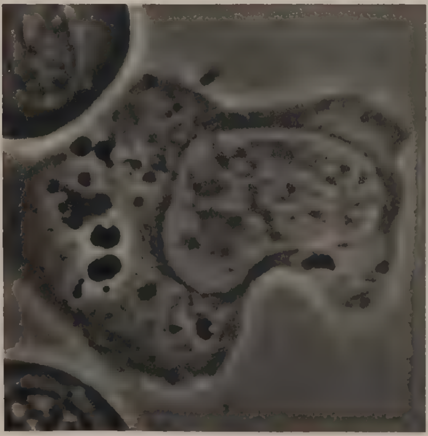

FIG. 6

HEMATOLOGY OF HEALTHY AND VIRUS-DISEASED SOCKEYE SALMON, ONCHORHYNCHUS NERKA

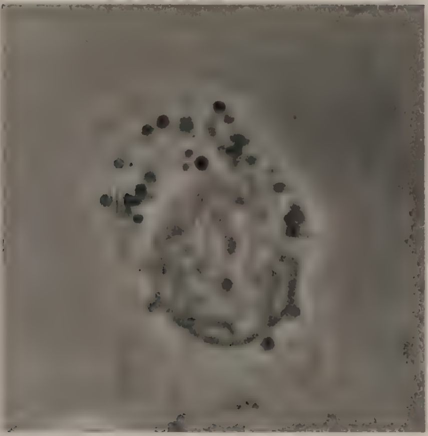

FIG. 7

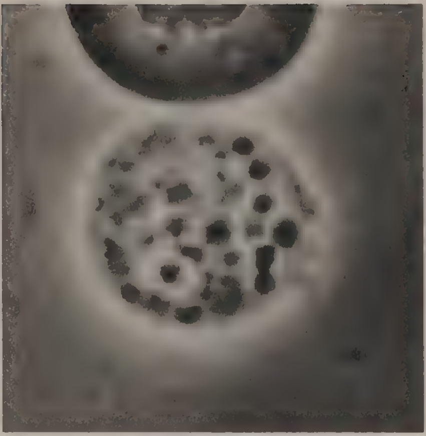

FIG. 8

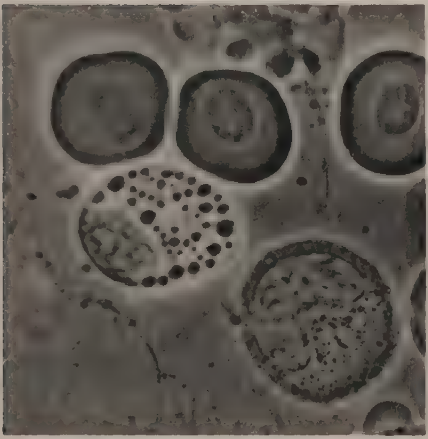

FIG. 9

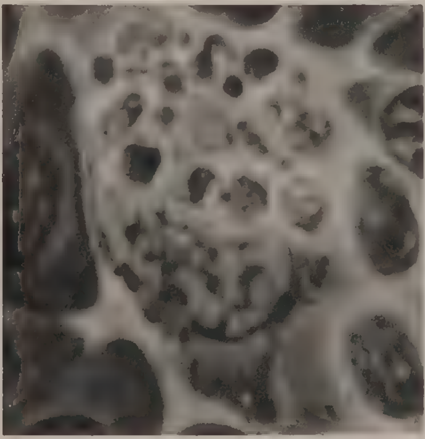

FIG. 10

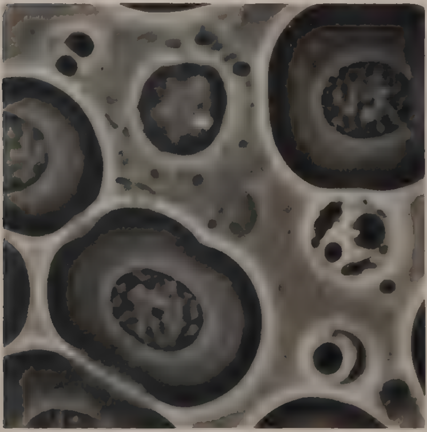

FIG. 11

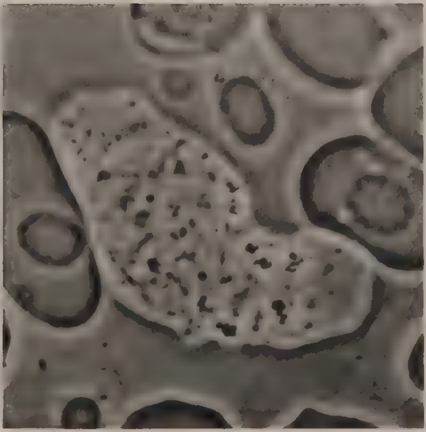

FIG. 12

HEMATOLOGY OF HEALTHY AND VIRUS-DISEASED SOCKEYE SALMON, ONCHORHYNCHUS NERKA

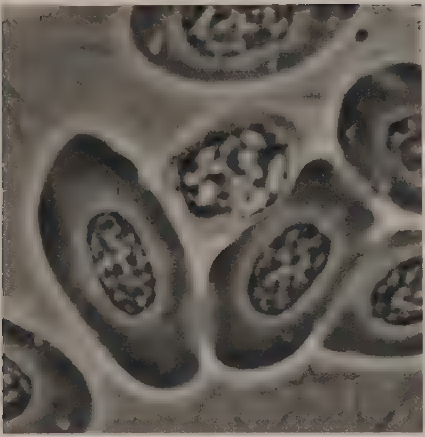

FIG. 13

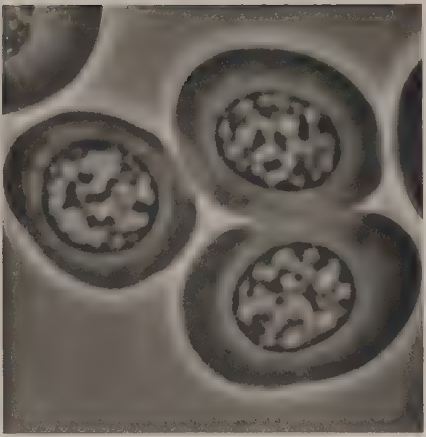

FIG. 14

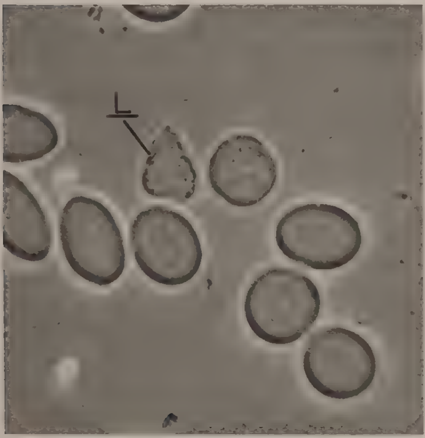

FIG. 15

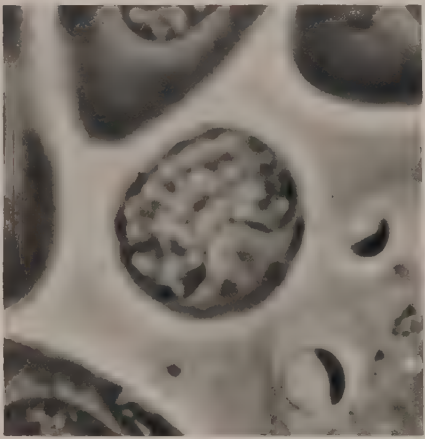

FIG. 16

# THE OCCURRENCE OF *GYMNODINIUM BREVIS* IN THE WESTERN GULF OF MEXICO

*By*

W. B. Wilson and S. M. Ray

## THE OCCURRENCE OF *GYMNODINIUM BREVIS* IN THE WESTERN GULF OF MEXICO

*Gymnodinium brevis* Davis has been associated with mass fish kills in the Gulf of Mexico near the west coast of southern Florida since 1947, but has never been reported from other locations. Between 1948 and 1950 the U. S. Fish and Wildlife Service maintained a laboratory at Sarasota, Florida, and although numerous samples were collected throughout large areas of the adjacent Gulf waters during this period, *G. brevis* was not observed. In November 1952, a bloom of *G. brevis* which occurred in an area south and southwest of Sanibel Island was investigated by U. S. Fish and Wildlife Service personnel aboard M/V *Alaska.* From September 1953 to January 1955, *G. brevis* was found consistently in one location or another along the Florida west coast and since January 1955, it has been found sporadically and in low concentrations in that area.

During the period 1953-55, sea water samples were collected at Galveston, Texas and along the Gulf coast between Galveston and Florida, but no *G. brevis* were observed.

In September 1955, we investigated a fish kill near Port Isabel, Texas. Twelve samples were collected from an area of the Gulf of approximately 20 square miles between the mouth of the Rio Grande and a point approximately 10 miles north of Port Isabel. All of the samples contained *G. brevis* with concentrations ranging from 50 to 500 per milliliter. A sample collected near Port Isab in Laguna Madre did not contain *G. brevis.* Fish we not dying at the time of our investigation, but dead fi extended along the Gulf beaches from the mouth of t Rio Grande to a point 17 miles north of Port Isabel.

C. E. Dawson and Henry Hildebrand at the Institu of Marine Science checked to a point 45 miles south the Rio Grande along the Gulf Beach of Tamaulipas air on September 15, 1955. Fish were observed dying a floating ashore in great numbers about 40 miles south the Rio Grande. On the following day they checked miles of beach from Playa Washington south and fou dead fish in large quantities along 21 miles of beach. water sample collected as a point 36 miles south of t Rio Grande contained a minimum of 22,000 *G. brevis* p milliliter. Information from fishermen and airplane pilo indicates at least 120 miles of coastline were affected the state of Tamaulipas. Fish were still dying on Se tember 27, 1955 along the Tamaulipas coast.

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# CEROID IN FISH *

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Since the original description of ceroid in rats,[1,2] many papers have appeared on the etiology and characteristics of this pigment. It was first seen as a yellow, granular pigment in hematoxylin and eosin sections of the cirrhotic livers of choline deficient rats. The pigment was more fully characterized by Endicott and Lillie,[3] and additional staining reactions were summarized recently by Lillie.[4] The pigment is sudanophilic in paraffin sections, acid-fast, basophilic, isotropic, iron negative, and highly resistant to solution in water, alcohol, fat solvents, and dilute aqueous acids and alkalis. It is stained by Mallory's hemofuscin stain and Weigert's myelin stain. It reduces osmium tetroxide and diamine silver carbonate but not ferric ferricyanide. The Gmelin reaction for bile pigments is negative. It has a greenish yellow fluorescence at 3650–3660 Å. It is Schiff positive with or without antecedent diastase digestion after performic or periodic acid oxidation.

Ceroid originally was considered a characteristic and specific microscopic finding in experimental hepatic cirrhosis of dietary origin.[5] Later, however, it was established that dietary cirrhosis could be produced without ceroid and that cod-liver oil enhanced the formation of the pigment.[6] Endicott[7] reported that prolonged *in vitro* oxidation of cod-liver oil or linseed oil by potassium bichromate produced acid-fast material similar to ceroid. He noted that these oils, when injected subcutaneously into rats, gradually acquired acid-fastness. Victor and Pappenheimer[8] confirmed that dietary cirrhosis unaccompanied by pigmentary deposits could be produced. They noted that ceroid was not dependent either upon fatty changes in liver cells or upon cirrhosis and that vitamin E retarded the appearance of the pigment. They described ceroid for the first time in humans, in 4 cases "of profound nutritional disorder."[9] They also found acid-fast pigments in many routine human necropsy cases. Similar pigments were found in 55 per cent of 38 cases of Laennec's cirrhosis. From their observations and a study of the literature, they concluded that ceroid was found in association with: (1) vitamin E deficiency of rats and other laboratory animals; (2) dietary cirrhosis in rats, and other cirrhosis of rats and other laboratory animals; (3) chronic biliary

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fistulae and experimental anemia in dogs; (4) nutritional disorders in man in which there was presumptive impairment of absorption by the small intestine; (5) cirrhosis of the liver and hemochromatosis in man; and (6) focal degenerative lesions. Mason *et al.*[10] reported on experiments with rats indicating that vitamin E, when added to diets rich in unsaturated glycerides, acted as an antioxidant, enabling the fat cells to incorporate and stabilize unsaturated fatty acids as "storage fat."

By 1950, ceroid had been described in rats by many investigators under a variety of experimental conditions usually involving low-protein, high-fat diets and cod-liver oil or a deficiency of vitamin E. It had been seen in vitamin E-deficient guinea pigs,[11] and in dogs and humans.[9] Edwards and Dalton[12] described the pigment in mice following treatment with carbon tetrachloride, but Lee[13] pointed out that the pigment observed in rats and in mice was not identical in every respect and implicated cellular necrosis as an important factor in production of the pigment.

Unsaturated fatty acids were recognized as important etiologic agents in the production of ceroid and Casselman,[14] in a series of well conceived experiments, demonstrated the *in vitro* preparation of substances resembling ceroid by the auto-oxidation of unsaturated fats, fatty acids, or their esters. Similar reactions were not given by hydrocarbons, saturated fats, or fatty acids. The formation of these ceroid-like substances was inhibited by antioxidants. He concluded that whenever conditions are such that unsaturated fats accumulate in tissues to such an extent that a relative lack of biologic antioxidants results, auto-oxidation of the fats and their conversion to ceroid pigments are favored. In a later paper,[15] in support of this view, Casselman reported that ceroid production was greater on diets with fats of high iodine numbers than with those of low iodine numbers and that the addition of either methylene blue or vitamin E as antioxidants inhibited the formation of ceroid.

Some additional support for Casselman's work is apparent in the reports describing "yellow fat disease" in mink[16] and swine.[17] Both papers reported the formation of ceroid following the use of diets high in the unsaturated fatty acids of fish and both conditions were alleviated by feeding vitamin E.

The mass of data relating ceroid formation to unsaturated fatty acids, particularly those of fish products such as cod-liver oil, led naturally to the question of the occurrence of ceroid in fish. Lovern[18] characterized fish fatty acids as being of the common straight-chain monocarboxylic series with a chain length of 12 to 28 carbon atoms.

With increasing length of chain, the limit of unsaturation becomes progressively greater. In cod-liver oil the fatty acids are approximately 16 per cent saturated and 84 per cent unsaturated. One might conjecture that fish must have a highly efficient method for preventing auto-oxidation of these unsaturated fatty acids or they would be particularly prone to the formation of ceroid. Cumings[19] reported on the effects of vitamin E deficiency in the guppy, *Lebistes reticulatus*. His account did not mention the formation of ceroid-like pigments. Pickford,[20] however, in studies of hypophysectomized killifish, *Fundulus heteroclitus*, described ceroid in both experimental fish and in normal wild fish, suggesting that the occurrence of the pigment may be normal in this species. She also described heavy accumulation of ceroid in certain experimental fish, which was attributed to a possible dietary deficiency or unidentified infection.

Since its completion in 1953, the Salmon Nutrition Laboratory (U. S. Fish and Wildlife Service, Cook, Washington) has been a center for the histopathologic examination of diseased fish from all parts of the United States. During the first year of operation, various fish diseases were examined in several different species. It early became apparent that a yellow granular pigment was present in many hematoxylin and eosin sections. It was of interest, therefore, to determine the relationship of this pigment to ceroid and its incidence in diseased fish.

## Methods and Materials

All tissues were fixed in Bouin's solution, stored in 65 per cent alcohol, and embedded in paraffin. Staining methods were selected from the paper by Endicott and Lillie.[3] Sections were stained for 1 hour in 1:1000 aqueous solutions of the acid dyes orange G, indigo carmine, and phloxine B; and for 1 hour in 1:1000 aqueous solutions of the basic dyes brilliant green, basic fuchsin, and crystal violet. The Ziehl-Neelsen carbolfuchsin stain was used to test for acid fastness. Sudan III and IV were used for examination of sudanophilia. The Lorrain Smith Nile blue sulfate stain for differentiating neutral fats also was utilized. The acid ferricyanide test of Perls was used for iron, and Schmorl's ferric chloride-ferricyanide test for Abnutzungspigmente. Weigert's Gram stain was used; and the Gmelin reaction was observed. The Schiff procedure was employed following performic and periodic oxidation, and without previous oxidation.[4] Tests for fluorescence were not made.

Ceroid-like pigments were observed in fish tissues from a variety of sources, as listed. Only 15 incidents are included in this report although the pigments were seen in sections from many more. Most of the specimens were received from the disease research station of the U.S. Fish and Wildlife Service Microbiological Laboratory, Kearneysville, West Virginia. Some of these represented specific diseases under experimental conditions, but others were single abnormalities observed in normal populations or moribund individuals from populations affected by unknown diseases. One group was selected for histologic examination from a nutritional experiment. Two were wild fish with external lesions. The others were various diseased fish from both State and Federal hatcheries throughout the United States. In each case in which pigment was observed in a diseased hatchery fish, non-moribund fish of the same species were sectioned to determine if the pigment was present. One fish with focal necrosis in the liver was included, although no pigments were observed in hematoxylin and eosin sections.

Dr. R. D. Lillie graciously submitted a sample of rat liver containing ceroid in phagocytes in areas of incipient scarring, for use as control material in the staining procedures.

Only sufficient information has been included in the case histories to demonstrate that the pigment was present in fish tissues under a wide variety of apparently unrelated conditions.

## BACTERIAL AND VIRUS DISEASES AND TUMORS

### *Hatchery Fish*

*Case 1.* Adult brook trout, *Salvelinus fontinalis,* with a natural infection of eastern kidney disease that is attributed to an unclassified gram-positive diplobacillus.[21] Pigment granules were observed in phagocytic cells in the spleen. (Fish and Wildlife Service, Berlin, N.H.)

*Case 2.* Fingerling brook trout with an experimental infection of eastern kidney disease. Pigment was scattered throughout the parenchymal cells of the liver, which showed no fatty change. (Fish and Wildlife Service, Kearneysville, W.Va.)

*Case 3.* Fingerling brook trout inoculated with Seitz filtrate of organs from fish infected with eastern kidney disease. Multiple areas of focal necrosis of unknown etiology were present in the liver, which was not fatty. No pigment was visible in hematoxylin and eosin sections. (Fish and Wildlife Service, Kearneysville, W.Va.)

*Case 4.* Fingerling rainbow trout, *Salmo gairdnerii,* with recurring bacterial gill disease probably caused by myxobacteria.[22] Pigment was scattered throughout parenchymal cells of the liver, which showed only slight fatty changes. (Fish and Wildlife Service, Kearneysville, W.Va.)

*Case 5.* Adult brook trout infected with ulcer disease caused by *Hemophilus piscium.*[23] Pigment was scattered throughout the parenchymal cells of the fatty liver and the spleen. (Fish and Wildlife Service, Kearneysville, W.Va.)

*Case 6.* Fingerling blueback salmon, *Oncorhynchus nerka,* infected with a virus disease causing over 90 per cent mortality.[24] Pigment was present in areas of focal necrosis in the liver, which was not fatty, and scattered throughout the spleen. (Fish and Wildlife Service, Leavenworth, Wash.)

*Case 7.* Adult guppy, *Lebistes reticulatus,* with large malignant hepatoma. Pigment granules were concentrated in foci of necrosis in both the liver and kidneys. The liver was extremely fatty and cirrhotic. (Fish and Wildlife Service, Kearneysville, W.Va.)

### *Wild Fish*

*Case 8.* Adult wild smallmouth bass, *Micropterus dolomieu,* infected with lymphocystis disease, probably of virus origin.[25] Pigment was scattered throughout the liver, which was slightly fatty, in phagocytic and parenchymal cells. (Fish and Wildlife Service, Kearneysville, W.Va.)

*Case 9.* Adult wild yellow sucker, *Catostomus sp.*, from the Shenandoah River where a high mortality occurred, probably due to a bacterial infection. Multiple areas of focal necrosis were observed in the liver, which was markedly cirrhotic but not fatty. Dense deposits of pigment were present in the necrotic areas. (Fish and Wildlife Service, Kearneysville, W.Va.)

## TOXIC MATERIALS

*Case 10.* Adult rainbow trout with an acute mortality caused by excreted metabolic products from too many fish in a limited water supply. Pigment was observed in small areas of focal necrosis in the liver, which was moderately fatty. (Fish and Wildlife Service, Kearneysville, W.Va.)

*Case 11.* Rainbow trout fry affected by an unidentified substance in the food or water. No fish pathogens were observed. Pigment was observed in the renal tubular epithelium. (Wyoming State Fish Hatchery, Auburn, Wyo.)

*Case 12.* Fingerling rainbow trout with a chronic disease having high mortality. A massive deposition of sudanophilic material was visible grossly in the liver. However, the liver was not infiltrated by fat. This condition and case 13 may be related to the gossypol content of cottonseed meal which was included in the diet.* (Fish and Wildlife Service, Kearneysville, W.Va.)

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* Communication from Dr. S. F. Snieszko, Kearneysville, West Virginia, April, 1955.

*Case 13.* Fingerling rainbow trout with a high mortality attributed to a toxic solvent residue or gossypol in cottonseed meal. Pigment granules were present in phagocytic cells in the spleen. The fish were markedly anemic. (West Virginia State Fish Hatchery, Petersburg, W.Va.)

### NUTRITIONAL DEFICIENCIES

*Case 14.* Fingerling rainbow trout on the following diet: fresh beef spleen, 40; fresh beef liver, 10; and dry feeds, 50 parts (fish meal, 45; cottonseed meal, 21; wheat middlings, 20; Standard Brands Type 50B yeast, 10; and sodium chloride, 4 parts). This diet appeared nutritionally adequate, growth was normal, and no symptoms of disease were present. A fine, granular material was observed in the hepatic parenchymal cells, which were moderately infiltrated with fat. (Fish and Wildlife Service, Kearneysville, W.Va.)

*Case 15.* Fingerling rainbow trout on the same diet as case 14 with the fresh beef spleen replaced by 11.1 parts of fish meal. Subnormal growth and a high rate of mortality indicated that this diet was nutritionally inadequate. No fish pathogens were observed. Pigment was scattered throughout the spleen. (Fish and Wildlife Service, Kearneysville, W.Va.)

*Case 16.* Ceroid in rat liver. (Dr. R. D. Lillie, National Institutes of Health, Bethesda, Md.)

## RESULTS

In 12 of the 14 sections containing ceroid-like pigments, staining reactions were observed nearly identical to those described for ceroid.[3] The exceptions follow. The acid-fast characteristic occasionally was somewhat variable. Most globules of the pigment were brilliantly acid-fast after prolonged decolorization (Figs. 2 and 3), but adjacent particles of pigment often were only a pale pink or colorless. The pigment also had a marked affinity for methylene blue which occasionally masked the acid-fast reaction. In these sections the material was light green in sharp contrast to the blue counterstain. Perls' acid ferrocyanide test for iron was negative in 9 of the 12 tissues. Three of the splenic tissues, cases 6, 13, and 15, gave a strongly positive iron reaction. In these sections there were globules of pigment present which were clearly iron-negative. However, other acid-fast and sudanophilic particles were iron-positive.

Without previous oxidation, the material was Schiff negative or, rarely, very faintly positive. It was strongly periodic acid-Schiff positive without antecedent diastase digestion (Figs. 5 and 7). Following performic acid oxidation, the pigment was Schiff positive but not as strongly as after periodic acid oxidation. The pigment in paraffin sections resisted prolonged treatment with water, ethyl alcohol, acetone, and xylene.

The staining reactions of the pigment in fish tissue were qualitatively similar to those of ceroid in the rat liver control (Fig. 1). Invariably, however, there was an intrinsic difference in the degree or intensity of staining. The fish pigments generally were stained with less intensity or with less uniformity than the rat ceroid.

The fish ceroid was morphologically similar to previous descriptions of ceroid by various workers. It existed in well defined, single globules and in a finely granular, more diffuse form (Fig. 5). The globular type was more strongly acid-fast. In fact, the globules of pigment were stained more intensely by all stains. The finely granular form was also acid-fast, albeit less so than the globular form. There was little to distinguish between the two forms by the Schiff test following performic or periodic acid oxidation.

Ceroid was found in the liver in areas of focal and diffuse necrosis. The pigment was observed also in liver sections which were not necrotic. In these sections it was found in parenchymal cells and in Kupffer cells. Two of the livers were cirrhotic and contained ceroid. In both cases, however, the ceroid was in focal areas of necrosis and not in the fibrous tissue. One of these livers was the site of a massive hepatoma. The ceroid, however, was not found in the tumor cells. The pigment was scattered throughout the spleen (Fig. 7). The granular type was observed more often in the spleen than in other organs and often appeared to be diffused throughout the reticular stroma. The pigment was observed in the basement epithelium of renal tubules in small, well defined droplets (Fig. 8).

In non-moribund hatchery fish of the same species as the diseased specimens in this report, ceroid was not observed.

Three of the 15 sections did not contain ceroid. In case 2, of eastern kidney disease, a pale yellowish to colorless pigment was observed throughout the parenchymal cells in hematoxylin and eosin preparations. This pigment was not acid-fast or sudanophilic. Case 3 was included in order to examine multiple areas of focal necrosis in the liver. No pigment was observed in these necrotic areas by any of the selected staining techniques. The liver of case 14 was from a fish on a nutritionally adequate diet, as indicated by growth. This section was markedly fatty, as is commonly observed in hatchery fish, and the cytoplasm of the parenchymal cells was markedly granular. These granules, however, gave no staining reaction similar to ceroid.

## Discussion

In mammals, ceroid generally has been seen only in rather well defined experimental conditions. Observations on human necropsy cases form the most notable exception to this statement.[9] In marked contrast, ceroid has been observed in fish in a wide variety of apparently unrelated diseases.

The ceroid observed in fish appears to be identical with that of mammals. It is likely that the iron-positive ceroid, which was observed in sections of spleen only, had combined with other hematogenous pigments. The ceroid of fish is similar morphologically to the ceroid of mammals, occurring in both globular and finely granular forms.

The pigment often is observed in areas of necrosis of the liver. However, necrosis is not necessary for its production in fish since the pigment has been observed without necrosis, and necrosis has been observed without ceroid. The spleen frequently contains ceroid but it has not been observed in conjunction with necrosis of that organ. There does not appear to be a direct relationship between simple fatty changes in the liver and the appearance of ceroid. The pigment was seen in livers densely infiltrated with fat and in livers with no readily observable fatty changes. Similarly, densely infiltrated fatty livers were seen unaccompanied by ceroid deposition. Cirrhosis was seen only rarely in these sections and apparently is not directly implicated in the production of the pigment in fish.

It appears that the species of fish reported here do not normally have ceroid in their tissues. However, nutritional deficiencies, bacterial and virus diseases, toxic products, and other physiologic disturbances readily result in the formation of the pigment. In Pickford's[20] report of ceroid occurring normally in wild killifish, the possibility of a chronic low-grade infection in the wild population cannot be excluded. Although hatchery diets usually have little in common with the food of fish under natural conditions, there is no indication that nutrition was an important cause of the ceroid observed in these fish. In many routine sections of non-moribund hatchery fish on diets producing normal growth, ceroid has never been observed. In the two wild fish in which ceroid was observed, lesions produced by bacterial or virus diseases obviously were present. We have not had an opportunity to examine non-moribund wild fish of these species.

These observations indirectly support Casselman's theory[14,15] that whenever unsaturated fatty acids accumulate in tissues so that a relative lack of biologic antioxidants results, auto-oxidation of the fats and their conversion to ceroid pigments are favored. It appears that fish, with their naturally high content of unsaturated fatty acids, are particularly susceptible to ceroid formation. The slight differences noted in staining intensity between fish ceroid and rat ceroid are probably related to differences in the composition of the unsaturated fatty acids of the two animals. There is no evidence that a deficiency of antioxidants was involved in the cases reported here. It is probable, however, that in situations of physiologic upset, a breakdown of transportation and enzyme systems could result in local deficiencies of essential nutrients.

This may well explain the occurrence of ceroid in focal degenerative lesions in other animals as well as fish. Little is known for fish of their vitamin E requirements or of those of other biologic antioxidants which they may utilize. This problem is now under investigation at the Salmon Nutrition Laboratory.

In view of the high incidence of ceroid in diseased fish, it is possible that this group of animals is unusually well suited for studies on the etiology of ceroid and possibly for other studies concerning the presence or absence of highly unsaturated fatty acids.

## SUMMARY

A pigment, apparently identical to ceroid, has been observed in fish affected with a wide variety of pathologic states, including nutritional deficiencies, bacterial and virus diseases, and disturbances due to toxic materials.

It appears likely that fish, with their high content of unsaturated fatty acids, are particularly susceptible to the formation of ceroid.

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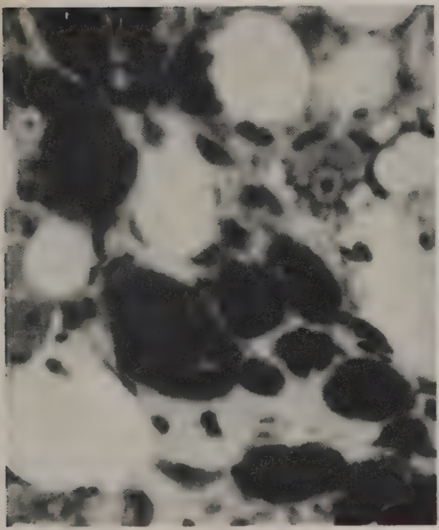
## LEGENDS FOR FIGURES

FIG. 1. Case 16. Ceroid in rat liver control. Acid-fast stain. × 500.

FIG. 2. Case 12. Ceroid in fish liver. Acid-fast stain. × 250.

FIG. 3. Case 12. Ceroid in fish liver. Acid-fast stain. × 500.

FIG. 4. Case 12. Ceroid in fish liver. Sudan IV stain. × 250.



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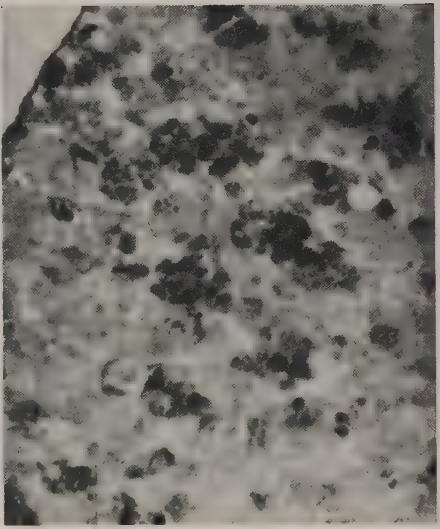

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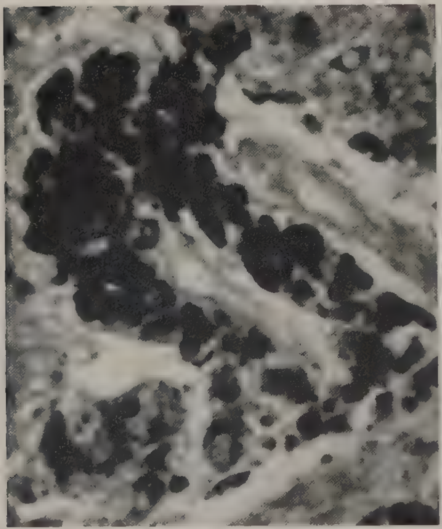

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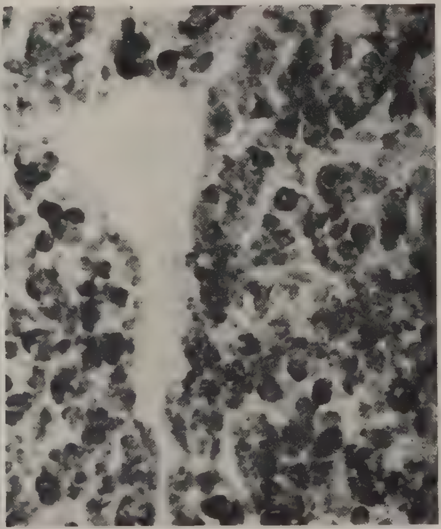

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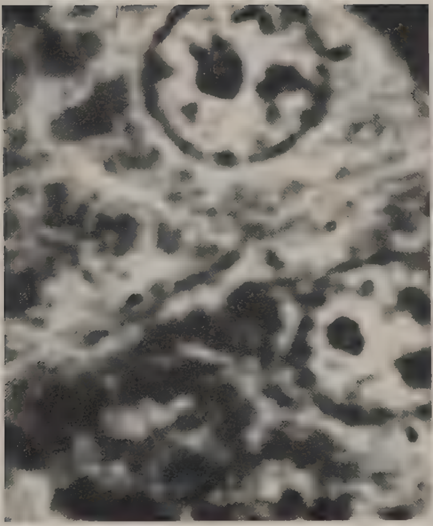
FIG. 5. Case 5. Ceroid in fish liver, showing a finely granular form. Schiff's stain after periodic oxidation. × 2,000.

FIG. 6. Case 7. Ceroid in fatty fish liver. Basic fuchsin stain. × 1,000.

FIG. 7. Case 6. Ceroid in fish spleen. Schiff's stain after periodic oxidation. × 2,000.

FIG. 8. Case 11. Ceroid in fish renal tubules. Sudan IV stain. × 500.



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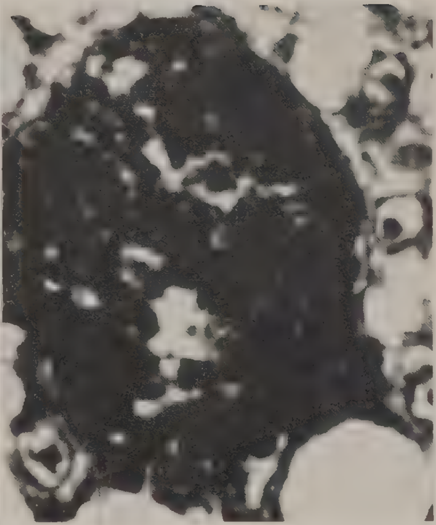

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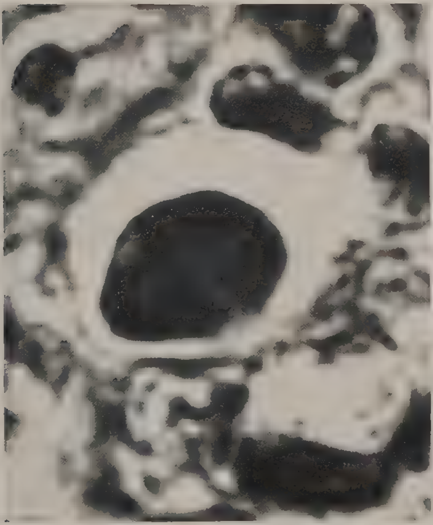

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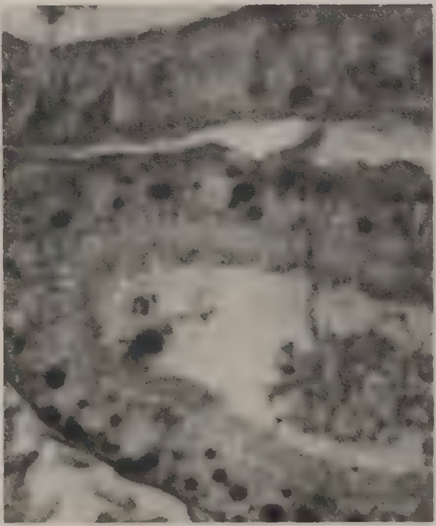

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# TISSUE DAMAGE IN SALMONIDS CAUSED BY *HALISIDOTA ARGENTATA* PACKARD

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During the histological examination of a collection of wild and hatchery salmonids, a peculiar foreign body was occasionally observed in various organs, particularly in the viscera. These objects, usually accompanied by a focal inflammation, were observed in 10 of 75 samples of wild trout and salmon collected in Oregon and Washington and were believed to represent an unknown type of parasitism. Their identity remained obscure until a massive concentration was observed in the tissues of wild coho salmon, (*Oncorhynchus kisutch*), from Minter Creek on the Olympic peninsula of Washington and in hatchery coho salmon from the Minter Creek Biological Station. The distribution of the structures suggested the intestinal tract as a point of origin. Subsequent stomach examinations revealed small, partially digested insect fragments with many long, spine-covered hairs. The insects were identified as second or third instars of the lepidopteran larvae, *Halisidota argentata* Packard. The spine-covered hairs penetrated the stomach wall and produced the observed lesions by working in an apparent porcupine quill-fashion throughout the body of the fish.

The pathology of the Minter Creek salmon was sufficiently extensive to merit a description of the condition for the benefit of other workers who may encounter this rather unusual phenomenon and confuse it with an actual parasitic infection.

## MATERIAL

Personnel of the Washington Department of Fisheries at the Minter Creek Biological Station collected the samples of wild and hatchery fish. These fish were fingerling salmon which had been feeding for 3 to 6 months. The samples were fixed in Bouin's solution for 24 hours and stored in 65% alcohol. Routine paraffin sections and hematoxylin and eosin staining techniques were utilized in preparing the material for examination.

## PATHOLOGY

### *Gross:*

When the abdomens of affected fish were opened and the viscera examined under a dissecting microscope, the larval hairs appeared as fine, brownish to yellow lines scattered throughout the viscera. Frequently large numbers of hairs were observed projecting radially from the outside wall of the stomach. When the stomachs were opened, it was usual to see a great many hairs penetrating the mucosa from which they could not be removed due to the spiny processes.

### *Microscopic:*

In hematoxylin and eosin sections, the hairs appeared as bright-yellow refractile foreign bodies surrounded by a narrow zone of inflammation. They were hollow structures without cellular detail. The inflammation was of a chronic type, usually consisting of small giant cells and monocytes. The inflammatory reaction was limited to the area immediately surrounding each individual shaft.

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However, there were frequently many hairs lodged in the visceral tissues and intense generalized inflammatory lesions resulted. This was particularly true in the adipose and pancreatic tissues among the gastric ceca. The spleen, which is usually located near the greater curvature of the stomach, was also punctured by multiple hairs. In various fish, however, almost every tissue was occasionally pierced by at least a single shaft. The liver, kidneys, auricle of the heart, spinal cord, and musculature were all observed with lesions. In some instances, hairs were observed just under the dorsal epithelium, having traversed the entire body musculature of the fish.

The hairs varied greatly in size with an approximate average diameter of 10 microns and extended to several millimeters in length. The spines angled away from the butt of the hair toward the tip and thus required a butt-first entry into the stomach mucosa.

## DISCUSSION

The literature records few, if any, examples of animal pathology of this type. It is probable that the incidence among wild fish is usually low, but in the occurrence described at Minter Creek a heavy concentration of the larvae was unquestionably present. In addition, the presence of similar hairs in over 10% of 75 wild samples indicates that the potential source of the injurious hairs is quite widespread. The hairs have been observed in cutthroat trout, (*Salmo clarki*), brook trout, (*Salvelinus fontinalis*), rainbow and steelhead trout, (*Salmo gairdneri*), brown trout, (*Salmo trutta*) and coho salmon. The larvae apparently are only rarely available to hatchery fish since they were observed only once in 75 samples.

Our material does not permit a quantitative evaluation of the tissue damage. However, the intense concentration of these structures in many fish, and the occasional penetration of vital organs makes it highly probable that some mortality is a direct result of the hair penetration.

## SUMMARY

Marked tissue damage was observed in young fingerling salmon from the Minter Creek Biological Station. The lesions were caused by plumose hairs from the larvae of *Halisidota argentata* Packard which penetrated the stomach and pierced the visceral organs and tissues of the fish. Similar hairs were observed in 10 of 75 samples of wild trout and salmon.

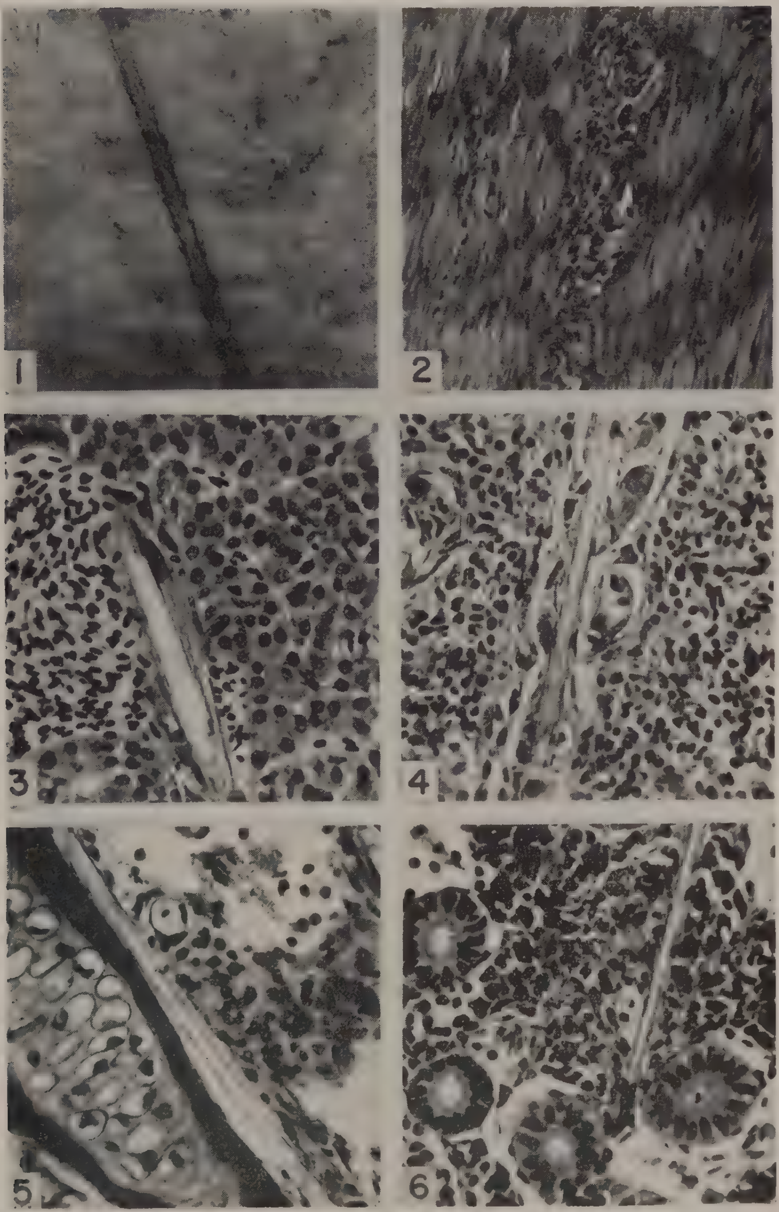
## ACKNOWLEDGEMENTS

We wish to thank Dr. Julius Rudinsky and Mr. John D. Lattin, Department of Entomology, Oregon State College for identifying the insect fragments, and Mr. Richard E. Noble, Washington Department of Fisheries, for collecting the fish samples from Minter Creek.

### Explanation of Plate
(All figures × 400)

Figure 1. A wet mount of a hair lodged in the mesentery.
Figure 2. An inflammatory zone surrounding a hair in the wall of the stomach.
Figure 3. A hair lodged in the liver.
Figure 4. A hair lodged in the spleen.
Figure 5. This hair pierced the vertebral column and is adjacent to a vertebra.
Figure 6. A hair lodged in the kidney.

## Plate I



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# HISTOPATHOLOGIC CHANGES OF A VIRUS-LIKE DISEASE OF SOCKEYE SALMON

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Rucker *et al.*, (1953) described a disease of sockeye salmon (*Oncorhynchus nerka*) of possible viral etiology. First seen in Washington in 1951 with relatively minor losses, the disease recurred in 1952 killing over two million fingerling salmon with a mortality rate of 91.5 percent (Watson, 1954). In 1953, the disease was present in every sockeye salmon hatchery in the state. Rucker, Watson and their associates have demonstrated that the disease is infectious, caused by a serially-transmissible and filterable agent, and specific for one species of fish. Watson *et al.*, (1956) have described the hematology of infected salmon. The present paper deals with the histopathology of the disease.

## Symptoms and Gross Pathology

Rucker *et al.*, (1953) described diseased fish as lethargic. The abdomens were distended and certain fish had hemorrhagic areas in some of the fins, at the base of paired fins or in the isthmus. The gills were pale, the spleen was light in color and the stomach was distended with a milky fluid. The gallbladder was filled with green bile, the intestine contained a watery, straw-colored fluid, and the large intestine was often red and apparently inflamed. Fluid expressed from the anus was yellowish and tinged with blood. There was some muscular necrosis in the anal region. Approximately 0.1 percent of the original populations eventually developed spinal deformities resembling scoliosis and lordosis.

Watson *et al.*, (1954) also noted the lethargy. They described, however, great excitability which occurred in about 10 percent of the affected fish. These individuals swam in circles on one side or the other and occasionally exhibited spasms of wild, excited swimming which left them exhausted on the bottom of the trough. Fish which succumbed at the start of an epizootic showed few or no external lesions. As the epizootic progressed, however, hemorrhagic areas similar to those noted by Rucker were also observed. Later there was a marked decrease in moribund fish with hemorrhagic areas. After 85 percent of the population was dead, spinal deformities were seen in some of the remainder.

Typically, the mortality rate rose to one percent per day within 6 to 9 days after the onset of the disease. The cumulative mortality approximated 50 percent within 16 to 22 days and 90 percent of the fish were dead within 25 to 40 days (Watson *et al.*, 1954).

## Microscopic Pathology

*Methods and Materials:* Fifty moribund fish were selected from an epizootic in an advanced stage as evidenced by a cumulative mortality over 80 percent, absence of surface hemorrhage and presence of spinal deformities. Tissues were fixed in Bouin's solution for 24 hours and stored in 65 percent alcohol. Sections were stained routinely with hematoxylin and eosin, Wolbach's Giemsa variant and Warthin's spirochete technique.

A number of stains, as noted, were used for the identification of endogenous pigments. Wright-stained blood smears were prepared from each fish.

*Microscopic:* Lesions were observed in the liver, spleen, brain, eyes, muscles, air bladder and intestine with a varying degree of incidence. A causative agent was not seen. No inclusion bodies, rickettsia, bacteria or spirochetes were observed.

*Liver:* A peculiar type of focal necrosis was observed in the livers of approximately 90 percent of the moribund fish. The lesions were characterized by necrotic parenchymal cells engorged with a yellow pigment. The size of the lesions varied from areas of one or two cells to large areas covering several adjoining lobules. The nuclei of affected cells were shrunken and twisted to abnormal shapes, usually forced to the periphery of the cell, and occasionally assumed half-moon formations. Generally, an entire cord of liver cells showed the same degree of change. Such an area retained the peripheral outline of a liver cord but the individual cell boundaries were masked to varying extents by masses of pigment. There was no inflammatory response or cellular debris which would indicate karyorrhexis. Cords of cells directly adjoining areas of the most advanced change appeared normal in many cases. In extensive areas of necrosis, active regeneration was present. No cirrhosis was observed. The lesions were irregularly distributed and were observed in both portal and central areas. Bile ducts appeared to be unaffected by the condition and were occasionally surrounded by necrotic liver tissue. The areas of necrosis were diffusely distributed but even in the most advanced cases not more than 10 percent of the total area of a section was involved.

The pigment in the necrotic cells was morphologically similar to the yellow, coarsely granular ceroid of choline deficient rats with hepatic cirrhosis (Lillie *et al.*, 1941). Numerous special staining techniques were used to confirm its identity. Like ceroid, it was sudanophilic in paraffin sections, acid fast, stained slowly by aqueous basic dyes, yellow by aqueous acid dyes, strongly stained by basic fuchsin, iron negative, gram negative, negative to Gmelin reaction and strongly Schiff positive after either performic or periodic acid oxidation. It was not soluble after prolonged periods in xylene, alcohols, dioxane or acetone. Therefore the pigment appeared identical to ceroid although tests for fluorescence were not made.

*Spleen:* In 90 percent of infected spleens, globular deposits of ceroid were observed. The pigment was seen in large macrophages but usually appeared to be deposited in sinusoids or intercellular spaces. There was no necrosis or degeneration in adjoining cells. The spleen was neither enlarged nor congested and there was no evidence of fibrosis. With the exception of the ceroid deposits, the spleen appeared normal.

*Brain:* In approximately 50 percent of the brains of infected fish

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EXPLANATION OF PLATE III

FIG. 1. Liver necrosis with ceroid deposition. H. & E. x 110.
FIG. 2. Ceroid deposits in the spleen. Basic fuchsin x 900.
FIG. 3. Lymphocytic meningitis. H. & E. x 100.
FIG. 4. Necrosis and inflammation of retina and vitreous body. H. & E. x 100.
FIG. 5. Extramedullary myelopoiesis in visceral fat. H. & E. x 100.
FIG. 6. Bilobed erythrocyte. Wright's stain x 400.

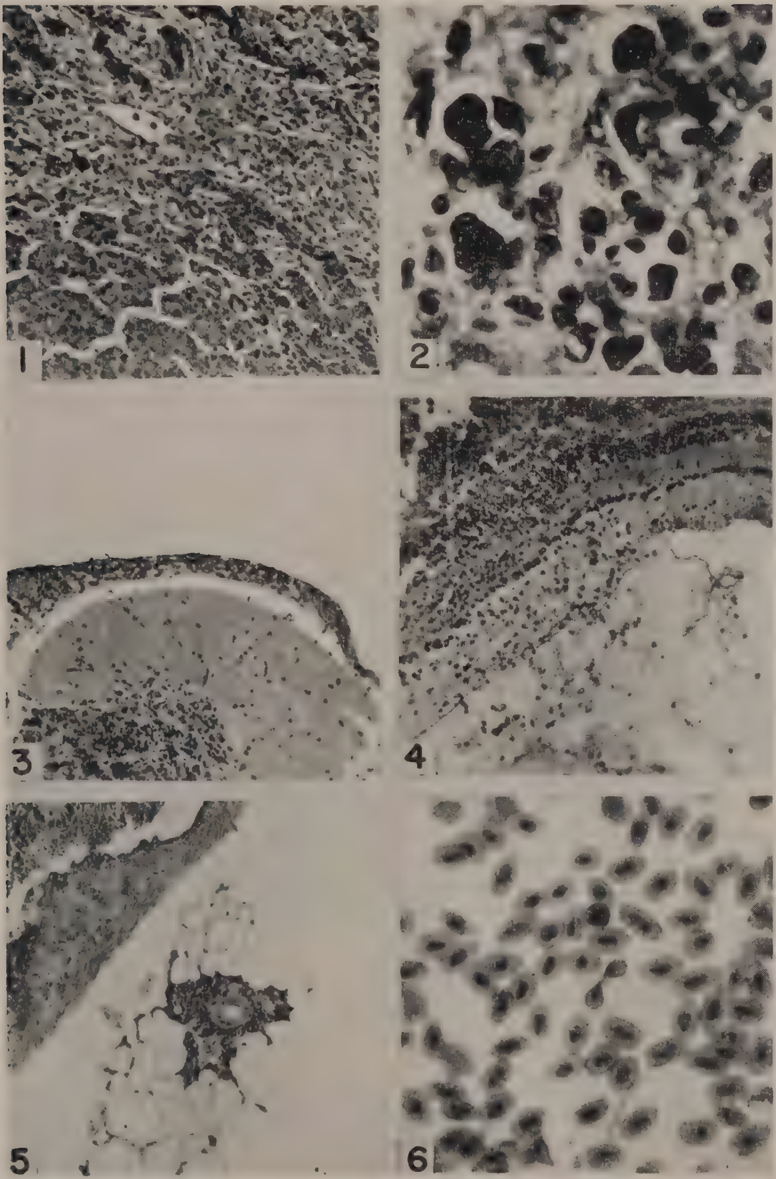

PLATE I

a mild meningo-encephalitis was present. The medullary meninges were most often and most severely involved but occasionally the lesion extended to the cerebral hemispheres. The encephalitis was confined to the brain stem and medulla. The cerebral hemispheres, cerebellum and olfactory lobes were not affected. The inflammatory reaction was diffuse in the meninges as evidenced by collections of lymphocytes and monocytes. In severe cases, small areas of necrosis were present in the brain stem accompanied by slight lymphocytic infiltration. These and areas of congestion were seen only infrequently. There was no perivascular infiltration.

*Eyes:* One or both eyes were affected by a mild inflammation associated with a polymorphonuclear infiltration in 30 percent of the infected fish. The iris and ciliary body were the most frequent sites involved although the retina was often affected at the anterior portion of the eye. Polymorphic leukocyte cells were also scattered throughout the posterior chamber of the eye in the vitreous humor. The cornea and lens appeared normal and there was no involvement of the optic nerve at the point of entry to the eye.

*Intestinal Tract*: Thirty percent of the infected fish had lesions of the intestinal tract. These consisted of small areas of focal necrosis almost entirely confined to the muscular layers and were scattered with approximately equal incidence from the anterior end of the tract to the anal opening. The epithelium was rarely involved.

Both healthy and diseased fish had a moderate amount of fat surrounding the viscera. This tissue was present in approximately 50 percent of the intestinal sections only. In 40 percent of these sections from moribund fish, extramedullary myelopoiesis was clearly present in small foci within the adipose tissue.

*Air Bladder:* The muscular wall of the air bladder was the site of a lesion closely resembling the condition observed in the muscular wall of the intestine. Small areas of focal necrosis involving the muscular components of the wall were noted in approximately 30 percent of the diseased fish.

*Muscle:* Ten percent of the muscle sections taken from the dorsal surface of affected fish showed a slight inflammatory reaction. In most cases only a small number of muscle bundles was affected. These were largely destroyed, leaving a vacuole which was occupied by polymorphonuclear leukocytes. The interstitial tissue was not involved. Since only one section, 2–3 mm by 3–4 mm, of dorsal muscle was sectioned from each fish, it is likely that the incidence of the muscle lesion was somewhat higher than observed.

*Blood:* Watson *et al.* (1956), with phase-contrast microscopy, have described two diagnostic characteristics in the blood of diseased fish. Before mortalities occurred, an intracytoplasmic, highly refractile body was observed in leukocytes and thrombocytes. When mortalities reached a peak, extracellular basophile granules and degenerated leukocytes and thrombocytes were present. Later stages of the disease were characterized by large numbers of immature erythrocytes, some of which were undergoing mitosis.

An abnormal blood picture was present in 90 percent of the diseased fish in the present study. A majority of the erythrocytes was immature and there was characteristically an abnormal cell present. This cell was a bilobed erythrocyte in the process of amitotic division. In numerous cases, the nucleus was entirely separated with only a thin strand of cytoplasm connecting the two daughter cells. Intermediate stages of mitosis were not observed but it appears likely that this was the same condition described by Watson *et al.* (1956).

*Other Tissues:* No pathologic alterations were observed in the following tissues: spinal cord, tongue, gills, heart, kidney, gallbladder, testes and ovaries.

## Discussion

The fish examined in this study potentially had been exposed to the disease for a relatively long period and might be considered chronically affected. It is possible that the lesions observed were more extensive than would be the case in fish which had succumbed quickly at the beginning of an epizootic. The lesions described are probably, but not necessarily, typical of the earlier phases of the disease. A decreasing virulence of the virus has been observed as the course of the disease progresses (Watson *et al*, 1954). It is likely that the observed lesions were more chronic than in fish which die in the early stages of the epizootic.

As in many virus disease no specific pathologic feature, such as the presence of inclusion bodies, was observed that would tentatively associate the causative agent with a virus. The failure to demonstrate other organisms, however, may indicate that the agent is sub-microscopic. It was not determined if the lesions observed were all due to the primary agent or to secondary infections which are common complications of virus diseases. However, when various organs of moribund fish were titrated in healthy fish, the highest concentrations of virus were found in the liver and kidney. The infectious agent was also demonstrated in the brain and spleen and, to a small extent, in the gills but was absent in the intestinal tract and muscle (Watson *et al.*, 1954). This appears to indicate that the intestinal and muscular lesions are not the result of direct viral activity. The bloody anal discharge described by Rucker (1953) could have originated from the muscular necrosis of the intestinal wall.

In view of the high virus titer, the failure to find pathological changes in the kidney was surprising. However, the fish kidney is the primary site of hematopoiesis. The concentration of virus in this organ is probably not linked to the renal system but is instead associated with the hematopoietic tissue. It is not clear whether the immature erythrocyte picture was a result of an interrupted maturation or was due to a marked stimulation of hematopoiesis. It is possible that extramedullary myelopoiesis was associated with the condition observed in the blood.

The occurrence of ceroid suggests an interesting field for further studies on the etiology of the disease. The relationship of labile methyl donors, alpha-tocopherol and cod liver oil to ceroid production has been described by many workers (György, 1944; Endicott *et al.*, 1944; Victor and Pappenheimer, 1945; Wachstein, 1945; György and Goldblatt, 1949; and Abell and Beveridge, 1951). Casselman (1951) has suggested that ceroid formation occurs whenever conditions favor the accumulation of unsaturated fatty acids in tissues to such an extent that a relative lack of biological antioxidants occurs. Fish normally have a very high content of unsaturated fatty acids. It would be of interest to determine whether ceroid deposition in this disease is related to a systemic deficiency of antioxidants, to merely a localized deficiency such as might occur as a result of focal necrosis or was directly related to the causative agent. In addition, the intracytoplasmic refractile body observed in leukocytes and thrombocytes by phase microscopy appeared pigmented under the light microscope (Watson *et al.*, 1956). Again, the relationship between this pigmentation, the ceroid of the liver and the spleen, and the agent are of concern.

## Summary

The histopathologic alterations of a virus disease of young sockeye salmon have been described. Microscopic lesions were observed in liver, spleen, brain, eyes, muscle, air bladder, intestines and blood. The disease is characterized by ceroid deposits in both the spleen and in areas of focal necrosis in the liver, meningo-encephalitis, a polymorphonuclear leukocytic inflammatory reaction in the eye, focal necrosis in the muscle wall of the intestine and air bladder, extramedullary myelopoiesis in the visceral fat and an abnormal blood picture.

No inclusion bodies, rickettsia, bacteria or spirochetes were observed. No pathologic alterations were observed in the spinal cord, tongue, gills, heart, kidney, gall-bladder, testes and ovaries. The significance of the observed ceroid deposition is discussed.

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# HISTOPATHOLOGY OF KIDNEY DISEASE IN FISH *

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Kidney disease is one of the most puzzling fish diseases known to exist in the United States. In less than 10 years it has invaded the Pacific Northwest, exacting a heavy toll of hatchery salmon. Its first appearance apparently was in Massachusetts where Belding and Merrill[1] described a disease similar to that now seen on the Pacific Coast. In 1946 it was diagnosed in Washington[2] and since that time has been observed in an ever increasing number of hatcheries. There are unpublished reports of the same or similar diseases in both California and Washington in the early 1930's.[3] The latest outbreaks occurred in the Federal hatcheries at Berlin, New Hampshire, and Cortland, New York, in brook, brown, and rainbow trout.[4] There is evidence to indicate that the disease may be much more widely spread in New York State.[5]

The disease is especially dangerous since little is known of the origin or source of the causative agent. Indeed, the classification of the diplobacillus associated with kidney disease is still uncertain. Thus, with our present knowledge, it is difficult or impossible to eradicate the malady from an infected hatchery.

Histopathologic studies were undertaken to clarify the pathology of the disease and to compare the eastern form with the western form.

## Materials and Methods

Fish exhibiting the typical lesions of kidney disease were selected from two eastern and two western hatcheries which were having active epizootics. Yearling and fingerling brook trout were available from the Fish and Wildlife Service stations at Cortland, New York, and Berlin, New Hampshire. Yearling coho salmon were received from the Oregon Fish Commission station at Sandy, Oregon, and yearling fall chinook salmon from the Fish and Wildlife Service station at Underwood, Washington.

These fish were fixed in Bouin's solution for approximately 24 hours, then stored in 65 per cent alcohol. Paraffin sections were prepared from the tongue, eyes, brain, gill, heart, dorsal and ventral muscles, kidney, liver, gallbladder, air bladder, spleen, and from the digestive tract at seven equally divided regions. The sections were

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stained with hematoxylin and eosin, Mallory's fungus stain, the Gram stain, and Wolbach's Giemsa variant.[6]

## HISTOPATHOLOGIC FINDINGS

Grossly, the disease has been characterized as a systemic infection with a pronounced affinity for the kidney.[2,4] Belding and Merrill[1] suggested that the complex disease picture was due, in fact, to impaired renal function.

Histologically, the disease was exemplary of the chronic granulomas. Productive fibrotic lesions were observed in every organ of infected fish. The proliferating fibroblasts characteristically formed distinct nodules, but often large masses of tissue occurred without tubercle-like structures. The granulomatous lesions apparently arose in the connective tissue stroma between the parenchymal cells of the various organs. Elements of the reticulo-endothelial system were frequently involved. Giant cells occurred, but only infrequently. As the lesions enlarged, they encroached upon and overwhelmed the parenchymal tissues (Fig. 1). In terminal cases, destruction of essential organs was tremendous. It was, in fact, remarkable that fish survived to the point of such extensive tissue damage.

The first organ to be affected by a natural infection of kidney disease was not determined with certainty, but it was quite likely that the hematopoietic tissue of the kidney was first involved. (In fish, the kidney is the major site of blood production.) In the earliest stage observed in this study, the hematopoietic tissue was always in an advanced stage of destruction. As the disease progressed, the posterior portions of the kidney were obliterated by the massive tissue reaction which apparently outstripped the vascular supply and terminated in necrosis and suppuration. In the anterior parts of the kidney, the granuloma usually progressed in a more orderly fashion, resulting in extensive fibrotic lesions which gradually enveloped the included renal tissue (Fig. 4).

Subsequently, or perhaps simultaneously, certain changes occurred in the digestive tract. From the esophagus through the small intestine, a marked eosinophilic inflammation occurred in the muscular wall. Eosinophils normally are present in the submucosal layers of the intestine. In moribund fish, their numbers were increased (Fig. 2). In the large intestine, the inflammation was granulomatous and involved the subcutaneous tissues in an extensive cellular reaction. The mucosal folds had a solid core of proliferating fibroblastic tissue (Fig. 3). There was also a characteristic cellular proliferation around the blood vessels of the intestinal tract, which was probably related to their endothelial elements.

The later stages of the intestinal lesions were characterized by an extensive peritonitis in the region of the gastric ceca. At first acute, with many inflammatory cells, both lymphocytic and polymorphonuclear, the lesions spread throughout the visceral fat and pancreatic tissue. The inflammation continued to the chronic granulomatous stage (Fig. 5), although sometimes it was replaced by massive necrosis affecting the entire pancreatic system (Fig. 6).

Cytoplasmic inclusion bodies were seen commonly in the pancreatic acinar cells. These inclusions did not resemble any described in the mammalian literature nor were they similar to those of infectious pancreatic necrosis.[7] They were round or ovoid, were often of tremendous size, forcing the nucleus to one side of the cell, and were strongly eosinophilic (Fig. 7). The larger forms sometimes contained basophilic globules or were uniformly basophilic (Fig. 8). The eosinophilic portions were gram-negative; the basophilic portions, gram-positive.

In the liver, granulomatous nodules formed in the connective tissue stroma between the cords of hepatic cells. These increased in size, forcing the liver tissue aside (Fig. 9). Actual necrosis of the parenchymal cells appeared minor but the hepatic tissue was gradually replaced by the proliferating inflammatory tissue. Cytoplasmic inclusions also were present in the parenchymal cells. These inclusions were most numerous at the periphery of necrotic areas (Figs. 10 and 11). When necrosis was absent, they were found adjacent to the granulomatous lesions in reduced numbers. Such inclusions were usually gram-negative, occasionally contained gram-positive globules, and were observed rarely with a high concentration of gram-positive diplobacilli. The liver inclusions appeared morphologically identical with the inclusions of the pancreatic acinar cells.

The splenic tissue was gradually replaced by the proliferating tissue of the granuloma until its normal structure was altered beyond recognition (Fig. 12).

As the disease entered the terminal stages, no tissue or organ escaped partial destruction. The heart was often the seat of a massive myocarditis with destruction of the major portion of the muscle (Fig. 13). The gills were enveloped in an inflammatory reaction extending from the arches to the tips of the filaments. The cerebral meninges usually contained complete granulomatous nodules (Fig. 14). The brain itself occasionally had necrotic foci or rings of inflammatory cells surrounding minor blood vessels (Fig. 15). The gallbladder and air bladder were sites of distinct granulomatous nodules. The subcutaneous tissue behind the eyeball was similarly affected.

The muscle lesions were distinctive. In most cases, they did not penetrate the epithelium of the skin and thus were not visible externally. They consisted of great masses of chronic inflammatory tissue extending far into the underlying muscular tissue (Fig. 16). The muscle lesions were a consistent site for the multinucleate giant cells so characteristic of the granulomas. In muscle sections, necrotic muscle bundles surrounded by normal tissue were frequently observed.

The cellular location of the gram-positive diplobacilli was of considerable interest. Both eastern and western workers have described the organism as being both intracellular and extracellular.[2,4] In tissue sections, the bacilli were rarely found concentrated in the parenchymal cells of any organ. In the liver and pancreas, they were found occasionally in parenchymal cells in close association with cytoplasmic inclusion bodies. In contrast, the organisms usually were found in tremendous numbers, both intracellularly and extracellularly, in the granulomatous lesions. In these areas, it appeared that the intracellular forms might be the result of active phagocytosis by inflammatory cells (Fig. 17). Many cells of the kidney were filled with the organisms. The lymphoid character of this tissue was also suggestive that phagocytosis was responsible for the intracellular position. It is entirely possible, however, that a specific intracellular stage was present in the cells of the granulomatous tissue. The areas of eosinophilic inflammation of the intestine did not contain the organisms although the muscular wall and mucosal folds were frequent sites for extracellular forms.

With Wolbach's Giemsa variant, the diplobacilli were stained reddish purple. Usually, the inclusions in the liver and pancreas were stained uniformly blue by this method. However, when many such inclusions were studied, there appeared to be a range of forms varying from homogeneous blue to packed masses of the violet diplobacilli with all intermediate stages.

## Comparison Between the Eastern and Western Types

Histologically, the general character of the disease appeared nearly identical whether it occurred in western salmon or eastern trout. Occasionally, individual specimens were seen in which the acute phases of the disease had swept into massive necrosis with resulting death before the typical chronic stages of the granuloma developed. The disease in salmon appeared to be somewhat more acute than it was in trout. The diplobacilli appeared morphologically identical regardless of the species of fish infected. In some of the trout examined, the organism stained less intensely with Gram and Giemsa stains and appeared smaller than the western form. Also in some trout, extensive granulomatous lesions were present when diplobacilli were nearly completely absent. These observations tend to confirm the reports of Snieszko and Griffin[4] who observed lesions of kidney disease without the concurrent presence of the diplobacilli.

The primary differences between the eastern and western forms of the disease appeared in connection with a secondary lesion which was rather characteristic. The eastern disease was often associated with a mycosis-like granuloma.[4,8] This, in effect, appeared to result in a second superimposed granuloma of different origin. The mycotic granuloma was sharply differentiated from the primary lesions by the frequent occurrence of foreign body giant cells in association with yeast-like organisms. In the western form of kidney disease, giant cells were limited almost entirely to the muscle lesions and the mycotic organism was not observed.

In the coho salmon from the Sandy, Oregon, hatchery, there was a marked incidence of the salmon poisoning fluke *(Nanophyetus salmincola* Chapin). The metacercariae were encysted in practically all of the fish tissues with heaviest concentrations in the posterior kidney and heart. Histologically, there was no apparent correlation between the encysted organisms and the lesions of kidney disease. In some cases the metacercariae were in the centers of granulomatous lesions. At other times, however, they were observed in tissues which were unaffected by kidney disease. The salmon poisoning fluke was not observed in the fall chinook salmon from Underwood, Washington, and was, of course, absent from the eastern samples.

A round, spherical organism, tentatively identified as *Schizamoeba salmonis,* was present in the anterior digestive tracts of both eastern and western samples. At the Cortland station, fish fed synthetic diets apparently were not infected by this organism but were infected with kidney disease. It would, therefore, appear doubtful that a significant relationship exists.

## Discussion

The granulomatous character of the lesions of kidney disease indicates an organism of low virulence or, conversely, indicates a high degree of resistance by infected fish. Eastern workers have experienced difficulty in transmitting the disease systematically except by injection.[4] This may be due to the apparent balance in pathogenicity of the organism versus the resistance of the host. It appears that salmon may be somewhat less resistant than trout, which may explain why western workers have less trouble in transmitting the disease by feeding infective materials.

The complex and extensive morphologic changes are probably not a direct result of impaired renal function. The renal system is one of the later tissues to be affected by the disease. Instead, each organ is affected directly by the pathogenic organism with the resulting symptoms. The edema frequently seen in the disease is probably the result of a damaged circulatory system, as is especially evident in the mesenteric blood vessels.

At present, the classification of the diplobacillus found in kidney disease is still in doubt. The rickettsia responsible for salmon poisoning in dogs[9] has been suggested as the causative agent of kidney disease.[2,4] The inclusions observed strongly indicate that an intracellular stage is present in the life cycle of the diplobacillus. However, Earp *et al.*[2] maintained at least limited growth of the organism on media containing coagulated chicken eggs, indicating that the organism may be bacterial instead of rickettsial. More recently, on the basis of slides from dogs,* Snieszko[10] believed there is little, if any, likelihood that the salmon poisoning of dogs is related to kidney disease. In mammals, granulomas are rarely attributed to rickettsial agents. Such diseases as typhus, spotted fever, Q fever, and trench fever, all rickettsial in origin, have practically nothing in common histopathologically with the lesions of kidney disease. The Bartonella group, members of the order Rickettsiales, do in some cases produce a granuloma and have, in addition, a marked affinity for the reticulo-endothelial system. The diplobacillus is gram-positive, while the Rickettsia are gram-negative. The reactions of the two organisms with Giemsa's stain, however, are very similar. The question is not resolved and awaits further bacteriologic studies. The occurrence of inclusion bodies, their apparent relationship to the diplobacilli, and the presence of granulomatous lesions without diplobacilli also indicate that the disease is more complex than a simple systemic bacterial infection.

The significance of the secondary lesions is obscure. Several workers have suggested the salmon poisoning fluke as a carrier for the causative agent,[2,4] and Snieszko and Griffin[4] have theorized that there may be a correlation between incidence of the disease and concentration of carrier-parasites in the hatchery water supply. Since the salmon poisoning fluke has a very limited northwestern distribution, it is apparent that either it is not involved as a carrier or that more than one carrier exists. Aside from the host-parasite relationship, there is an additional likelihood that these supplementary pathogens are directly related to the incidence of kidney disease. Difficulty in transmitting the disease by contact and by feeding of infectious material is indicative of a high degree of resistance. It appears possible that these secondary pathogens may serve the important purpose of breaching the outer defenses of the fish and allowing entry to the disease organism. The possibility of such infection would be increased if the causative organism was present in other aquatic hosts. Brockway[11] has found morphologically similar, gram-positive diplobacilli in many aquatic animals, including the scud, salamander, mayfly, caddis fly, frog, chironimid, blood worm, and dragon fly larva. Even after the organism has gained access to the animal body, it apparently meets a strong resistance which culminates in death only after an extended period. The debilitating effect of the mycotic granuloma and of a massive infestation of the salmon poisoning fluke augments suspicion against these organisms even though they may be proved non-carriers of the kidney disease organism itself.

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* Kindly furnished to S. F. Snieszko by W. J. Hadlow, D.V.M., National Institutes of Health, Hamilton, Montana.

## Summary

Histologically, the eastern and western forms of kidney disease are nearly identical. In both salmon and trout, the disease is characterized as a systemic granuloma.

Cytoplasmic inclusion bodies are present in the liver parenchymal cells and pancreatic acinar cells. These inclusions may be a stage in the life cycle of the causative agent.

The Gram-positive diplobacilli, which stain intensely with Giemsa's stain, are concentrated primarily in the granulomas where they occur both intracellularly and extracellularly. They are rarely seen in parenchymal cells.

The occurrence of secondary parasites in fish infected with kidney disease suggests that these parasites may have a rôle in introducing the causative agent and/or in lowering the resistance of the fish.

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## LEGENDS FOR FIGURES

Fig. 1. Normal renal tubules surrounded by granulomatous tissue. Brook trout. Hematoxylin and eosin stain. × 200.

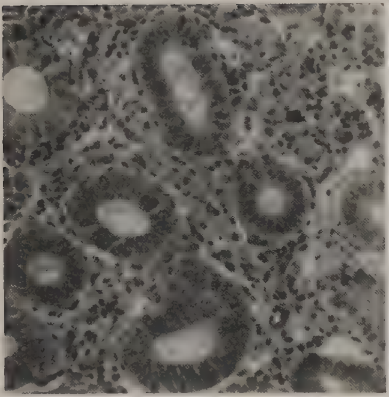
Fig. 2. Eosinophilic inflammation of the intestinal wall. Epithelium at top. Coho salmon. Hematoxylin and eosin stain. × 100.

Fig. 3. Granulomatous inflammation of large intestine. Brook trout. Hematoxylin and eosin stain. × 100.

Fig. 4. Necrosis and granuloma gradually engulfing the renal tubules. Coho salmon. Hematoxylin and eosin stain. × 100.

Fig. 5. Granulomatous tissue infiltrating the pancreas. Intestinal wall at lower left. Brook trout. Hematoxylin and eosin stain. × 100.

Fig. 6. Massive necrosis obliterating the pancreatic tissue. Gastric ceca at upper left. Chinook salmon. Hematoxylin and eosin stain. × 100.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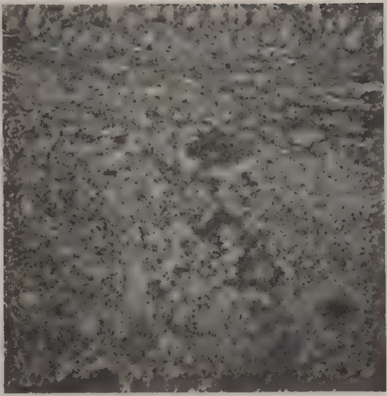
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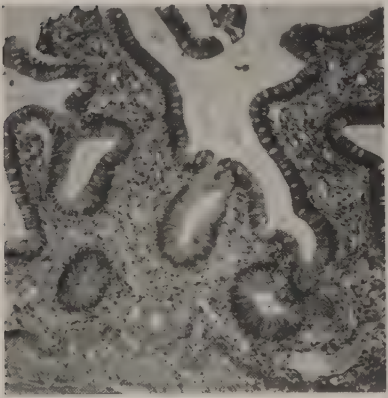
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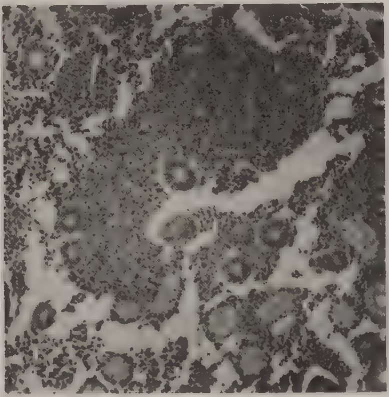
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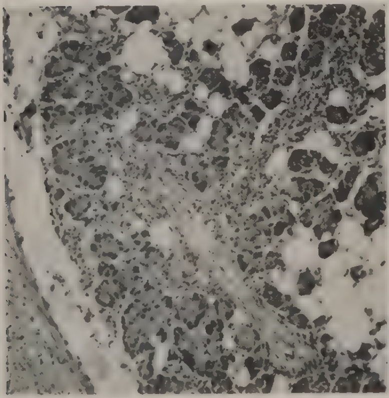
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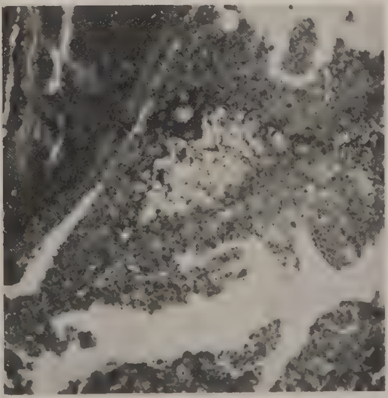
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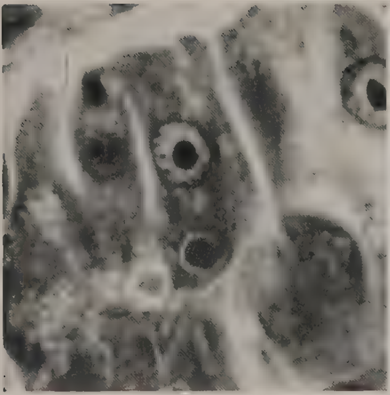
FIG. 7. An eosinophilic inclusion is present in the lower part of the pancreatic acinar cell in the center. Coho salmon. Hematoxylin and eosin stain. × 1350.

FIG. 8. From left to right, a lobed basophilic inclusion, an eosinophilic inclusion with basophilic granules, and an eosinophilic inclusion, all in pancreatic acinar cells. Coho salmon. Hematoxylin and eosin stain. × 1350.

FIG. 9. A granulomatous nodule, center, displacing the liver tissue. Brook trout. Hematoxylin and eosin stain. × 200.

FIGS. 10 and 11. Basophilic and eosinophilic inclusions in liver cells. Coho salmon. Hematoxylin and eosin stain. × 1350.

FIG. 12. The granulomatous proliferation has completely replaced the splenic tissue, making it impossible to discern the borderline between spleen and visceral fat. Brook trout. Hematoxylin and eosin stain. × 100.



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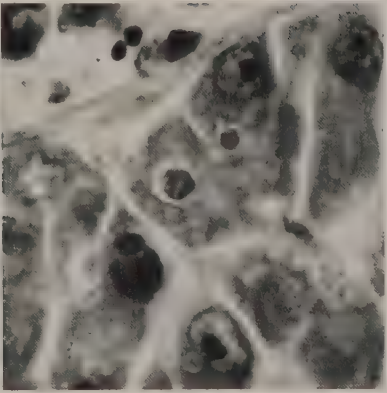

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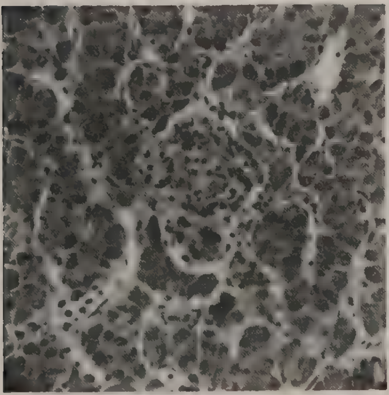

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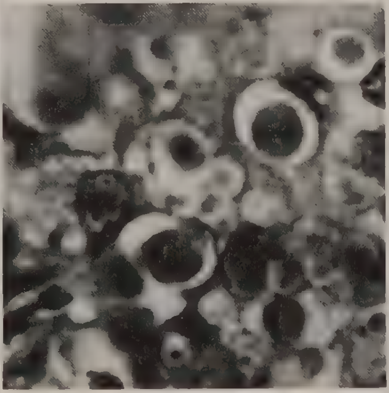

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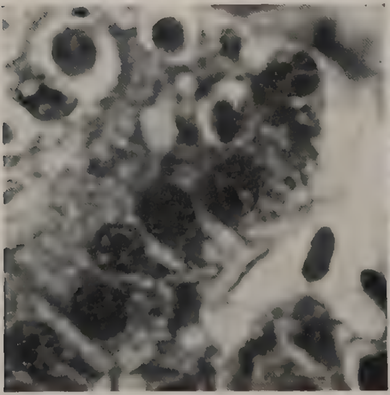

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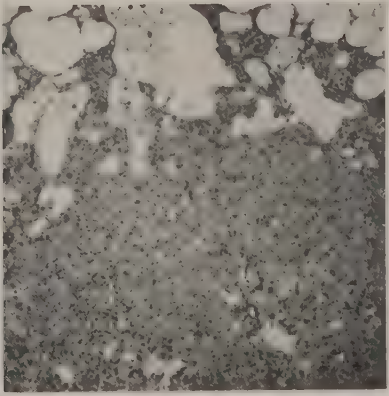

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FIG. 13. Granulomatous inflammation of the ventricle. Brook trout. Hematoxylin and eosin stain. × 100.

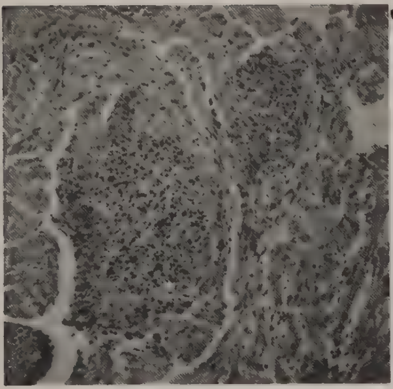
FIG. 14. Nodules of granulomatous tissue between the meninges and the brain proper. Chinook salmon. Hematoxylin and eosin stain. × 100.

FIG. 15. An inflammatory nodule and necrosis within the brain. Brook trout. Hematoxylin and eosin stain. × 100.

FIG. 16. The muscle lesion. Epithelium at top. Muscle myomeres are at right and left with the granulomatous lesion extending between them. Brook trout. Hematoxylin and eosin stain. × 60.

FIG. 17. Inflammatory cells filled with the diplobacilli, top center. Normal pancreatic acinar cells below. Coho salmon. Mallory's fungi stain. × 1350.

FIG. 18. Gram-positive diplobacilli. Brook trout. Gram stain. × 6000.



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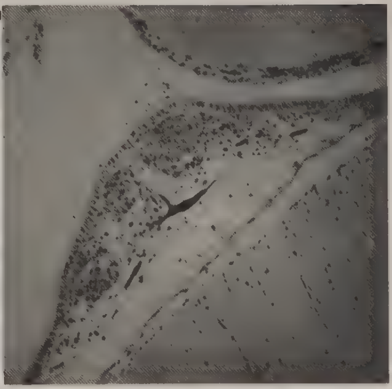

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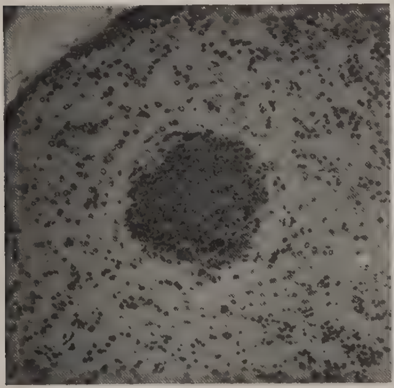

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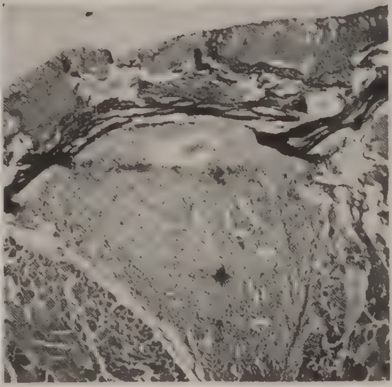

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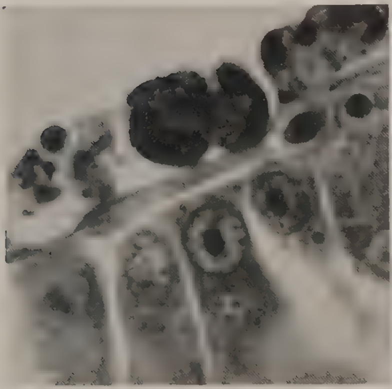

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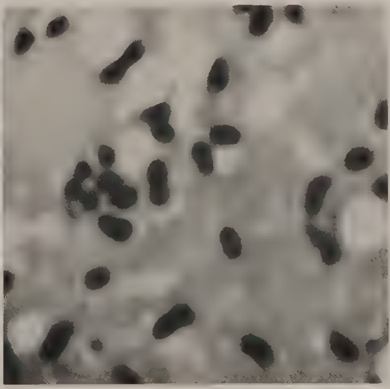

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# A MYCOSIS-LIKE GRANULOMA OF FISH

E. M. WOOD, W. T. YASUTAKE, AND W. L. LEHMAN

FROM THE U. S. FISH AND WILDLIFE SERVICE, SALMON NUTRITION LABORATORY, COOK, WASHINGTON, AND THE GOOD SAMARITAN HOSPITAL, PORTLAND, OREGON

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# A MYCOSIS-LIKE GRANULOMA OF FISH

E. M. WOOD, W. T. YASUTAKE, AND W. L. LEHMAN

From the U. S. Fish and Wildlife Service, Salmon Nutrition Laboratory, Cook, Washington, and the Good Samaritan Hospital, Portland, Oregon

Mycoses of systemic distribution are rarely observed in fresh-water fish in this country. In a recent review of atypical cell growths in fishes, Nigrelli[1] cited the only known instance of a mycetoma in a North American fresh-water fish which occurred in the head of fingerling landlocked salmon from an Idaho hatchery.[2] The fungus associated with this granuloma was characterized by a branching septate mycelium. Rucker[3] reported a streptomycete which was pathogenic to blueback salmon. This organism produced internal nodules containing masses of hyphae but no inflammatory response. A pathogenic fungus has been observed frequently in marine fish,[4] however, and in both marine and fresh-water fish in Europe. This organism was tentatively classified as a Phycomycete in or near the order Chytridiales and was assigned to the genus and species *Ichthyophonus hoferi*,[5] later reclassified as *Ichthyosporidium hoferi*.[4] The granuloma described in this report occurs in fresh-water trout and is apparently caused by a budding, yeast-like form with no hyphae which evokes a tremendous inflammatory reaction. Morphologically, the organism does not resemble the previously described Ichthyosporidium. The lesions were first seen accidentally in sections prepared from a diplobacillus infection of brook trout termed "kidney disease." Subsequently, the granuloma was observed in three widely separated infections involving the diplobacillus in each instance.

The histological material was received in a fixed condition; thus, no cultural data were available and the nomenclature and classification of the mycotic organism were not attempted. The present distribution of the disease, however, with its potential threat to domestic fish populations, seemed to warrant a description and discussion of the disease. Efforts are in progress to culture the organism.

## MATERIALS

During a 2 year period, the granuloma was diagnosed in tissue sections from fish hatcheries in New Hampshire, New York, and West Virginia. In each case kidney disease was present in epidemic form or had been introduced experimentally.

Tissues were taken from living, moribund fish and fixed in Bouin's solution for 24 hours, then stored in 65% alcohol. Hematoyxlin and eosin, Mallory's fungi stain, carbol fuchsin-methylene blue acid fast stain and gram stain were used for all sections.

Case 1.—In the spring of 1952 a serious mortality occurred in the brook trout population of the U. S. Fish and Wildlife Service Station at Berlin, New Hampshire. Individual ponds suffered losses over 50% of their total populations. The disease recurred in 1953 at approximately the same time of year. The mortality reached a peak in early spring and gradually subsided throughout the summer. Affected fish were sluggish, often had distended abdomens, exophthalmos and external blisters on the sides and belly.

Gross examination revealed two characteristic internal lesions. The kidneys were affected to a marked degree by a suppurative-necrotic process

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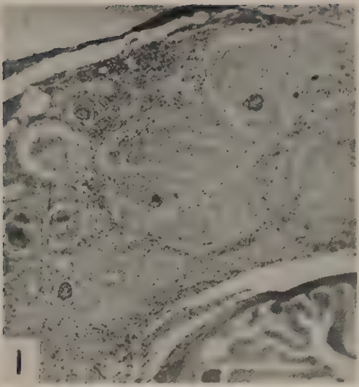
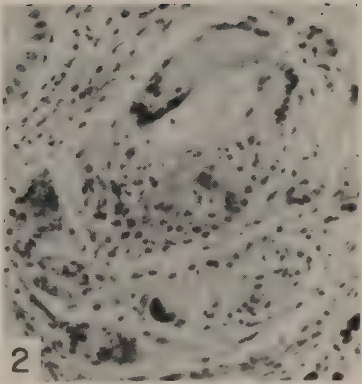
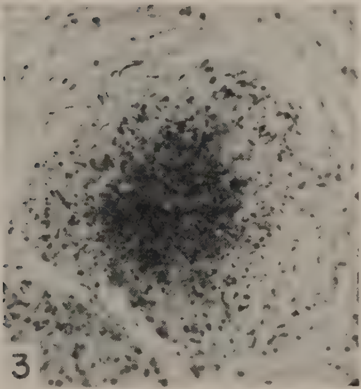
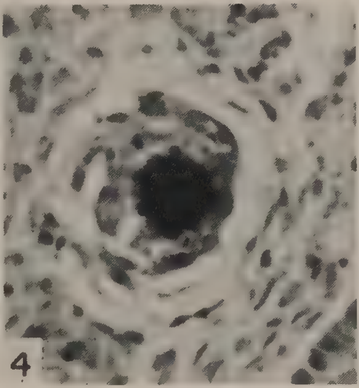
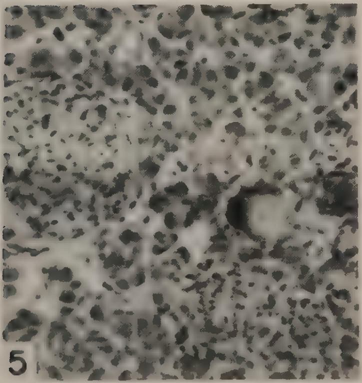










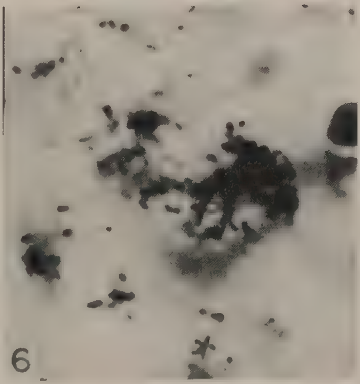


Fig. 1.—Mycotic granuloma. Intestine at lower right. All other tissue is aberrant. ×45.

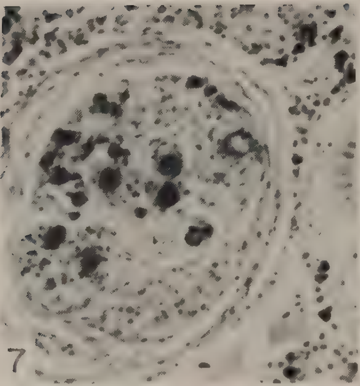
Fig. 2.—Tubercle in kidney with giant cells. ×200.

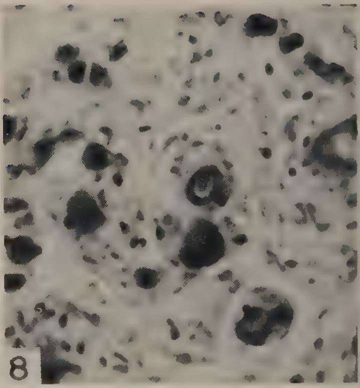
Fig. 3.—Typical abscess of visceral lesions. ×200.

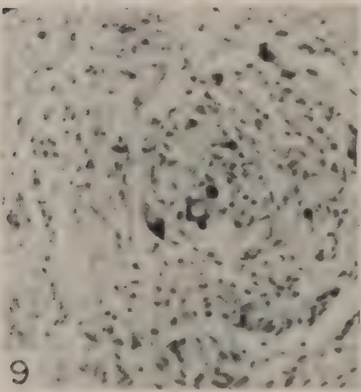
Fig. 4.—Small asteroid in center of tubercle. Mallory's fungi stain. ×400.

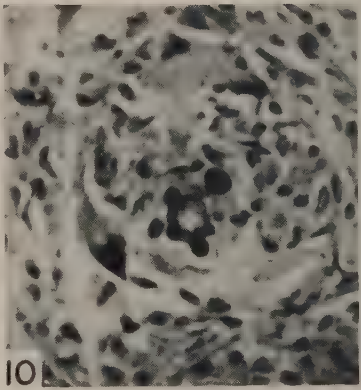
Fig. 5.—Granuloma in liver from case 2. ×200.

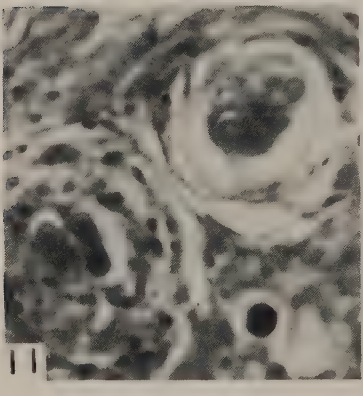
Fig. 6.—Diplobacillus in section at periphery of granuloma. Gram stain. ×2000.











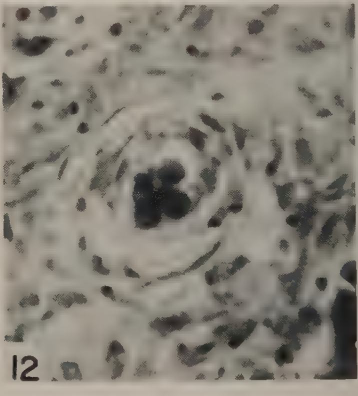


Fig. 7.—Tubercle in kidney with mycotic organism. ×200.

Fig. 8.—Figure 7. ×400.

Fig. 9.—Tubercle in kidney with mycotic organisms and giant cells. ×200.

Fig. 10.—Figure 9. ×400.

Fig. 11.—Mycotic organism in visceral lesion. ×400.

Fig. 12.—Mycotic organism in visceral lesion. ×400.

containing large numbers of gram-positive diplobacilli.[6] The second lesion was represented by a systemic granulomatous reaction most strikingly manifested by masses of aberrant tissue surrounding the gastric caeca and intestines or in extreme cases entirely filling the abdominal cavity. Neither lesion was observed in every moribund fish and both were seen in early stages appearing as slight inflammations.

The rate of mortality subsided after sulfonamide treatments. Gantrisin, sulfamerazine, and sulfamethazine were most effective. However, no treatment was effective in completely eradicating the disease.[6]

Case 2.—In early 1953 material was received from the U. S. Fish and Wildlife Service Experimental Station at Cortland, New York, for diagnosis of an experimentally induced choline deficiency. The granuloma was seen in sections of this material (including the controls) in trace amounts. There was apparently no correlation between the nutritional experiments and the incidence of the granuloma. The diplobacillus was not observed in these sections and no mortality was occurring in the experimental fish at that time. Three months later, the trout population at Cortland suffered a severe mortality. The kidneys were affected by a suppurative necrotic process containing large numbers of the diplobacillus. Grossly, there was no evidence of the granuloma. The large masses of aberrant tissue observed in the New Hampshire fish were not present. Histologically, however, there was an acute granulomatous lesion in the liver which contained the diplobacillus but not the mycotic organism. The mycotic organism was found, however, in trace amounts in the visceral fat surrounding the gastric caeca. Grossly, the disease resembled the epidemic at the New Hampshire Station. The fish exhibited the same symptoms, had approximately the same mortality, and the organism in the kidneys was morphologically similar to the diplobacillus seen previously.

Case 3.—An experimental program was conducted at the U. S. Fish and Wildlife Service Station, Leetown (P. O. Kearneysville), West Virginia, in 1953 to determine the effect of various sulfonamides and antibiotics on the diplobacillus found in the kidneys of diseased fish from Berlin, New Hampshire.[6] To produce a diseased population, individual fish were infected with the diplobacillus by intramuscular or intraperitoneal injection of a suspension of organs of infected fish and placed with the experimental groups. The disease was not transmitted, however, during a 3-week period. The experimental groups were then fed for one day a diet strongly enriched with ground brook trout which had just died or were about to die from kidney disease. Transmission was again unsuccessful. Subsequently, the fish were redistributed, 100 fish per group, and half of each group was infected by parenteral introduction of a freshly prepared suspension from trout with advanced kidney disease. These groups were then treated with various antibiotics. (The results of this series of experiments are published elsewhere.)[6] There was again no transmission of kidney disease to the uninfected half of each experimental group. However, in these uninfected groups subacute stages of the granuloma were present in every group regardless of the medication which they had received. The mycotic organisms were present in large numbers, particularly in the walls of the anterior part of the digestive tract.

The fish which were infected with kidney disease by injection resembled the fish with the natural infections at the New Hampshire and New York stations. The gross pathology and symptoms of the disease were identical and the diplobacillus was recovered from the internal organs of the fish in large numbers.

## HISTOPATHOLOGY OF THE GRANULOMA AND KIDNEY LESION

The granuloma, in the presence or absence of the diplobacillus, was typical of the granulomas associated with the various mycoses and tuberculosis of mammals. It was systemic in distribution. In advanced cases, the visceral lesions formed a white, glistening mass which was often mistaken for mature testes. The material was hard and granular, however, due to the tubercle-like nodules which formed a major part of the tissue. In earlier stages, these nodules formed barely perceptible papillae projecting from the outer walls of the stomach or esophagus. Lesions were observed in the muscular wall of the gastrointestinal tract, the adipose tissue surrounding the viscera, the gills, liver, spleen, kidney, eyes, and musculature. No lesions were noted in either the central or peripheral nervous systems. In case 3, which evidently represented a subacute stage of the disease, there was a marked specificity for the anterior portions of the gastrointestinal tract. The muscular walls of the esophagus and stomach were practically obilterated by the developing granuloma. Posterior to the juncture of the gastric caeca with the small intestine, there was no evidence of the lesion whatsoever. In this material, scattered lesions were observed in the kidneys and other organs but to a much lesser degree.

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[6] Snieszko, S. F. and Griffin, P. J. 1955, Prog. Fish. Cult. 17(1): 3–13.

The tissue reaction was granulomatous in character. Typical, well-defined tubercles were commonly seen. Giant cells were numerous. In the acute cases, polymorphonulcear leucocytes were the prominent cell type, but leucocyte reaction was negligible in the older, more chronic tissues. In one instance a very marked eosinophilic invasion was observed. When the production of tubercles and granulation tissue was intense in the visceral fat, typical microscopic abscesses were common. As pointed out by Baker[7] this characteristic is typical of blastomycosis.

The organism was a round, budding double-walled, yeast-like form, somewhat resembling the blastomycetes. A heavy capsule was sometimes observed. Endosporulation was not seen nor were hyphae observed. The size ranged from small, single, oval cells of 4 to 5 $\mu$ to large, budding cells up to 30 $\mu$. The affinity for hematoxylin was so variable that the darker staining individuals appeared calcified while many forms appeared as "ghosts." The same staining variability was observed with eosin in Mallory's fungi technique.[8]

In advanced cases, yellow, asteroid-like formations were frequently observed. Such asteroids were not stained by eosin but were heavily stained by basic fuchsin. They were rarely over 50 $\mu$ in diameter. High magnification failed to resolve any mycelial structures and no organisms were stained within the asteroids by Gram, acid fast, or Giemsa techniques.

When the diplobacillus was present, its location in relation to the granuloma showed considerable variation. In case 2, which was an extremely acute and apparently virulent strain, the bacillus was found throughout the primary stages of tubercle formation, particularly in the liver, but also in the kidney and other visceral organs. In an unusually chronic case from the West Virginia Station, however, the organism was rarely found within the areas of well-defined tubercle formation, but persisted at the periphery of such areas.

The kidney lesion in all cases appeared to be a combination of the granuloma and of a fulminating, massive necrosis attributed to the diplobacillus. In fish, the kidney is the primary site of hematopoiesis, and it was the hematopoietic tissue which was principally affected by the necrosis. The renal epithelium was apparently a secondary victim as the necrosis progressed. It was not unusual, however, to observe normal tubules and glomeruli totally surrounded by completely necrotic tissue.

### KIDNEY DISEASE AND THE GRANULOMA

Kidney disease is most prevalent on the Pacific coast of the United States in young salmon. The diplobacillus is morphologically similar to the organism observed in the eastern form of the disease. Earp et al[9] successfully cultured the organism in suspensions of minced chick embryo tissue. Subsequently, the organism was subcultured on Dorset's egg medium. No attempts have been made as yet in the eastern United States to culture the diplobacillus.

Snieszko and Griffin[6] have suggested

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[7] Baker, R. D. 1942, Am. J. Path. 18: 479–498.

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that the eastern and western forms of the disease are identical. Although the organisms of the two forms of kidney disease are morphologically similar, there are some characteristics of the diseases which differ. The western form appears to have a marked virulence in the fall and winter months during a falling water temperature. The eastern form, at least at the New Hampshire Station, is more virulent in the spring during a rising water temperature. Of interest to this discussion, is that the granuloma has not been reported in the western form of the disease by Earp and his associates. In a limited number of histological sections of the western disease, it has not been seen by workers at the Salmon Nutrition Laboratory. In contrast, no eastern form of the disease has been seen without the simultaneous occurrence of the granuloma.

## DISCUSSION

There is no experimental evidence to establish a correlation between the granuloma and kidney disease. Indeed it must be acknowledged that the identification of the granuloma as a mycosis is based on the similarity of this disease to those described in mammalian literature. However, the simultaneous occurrence of the two organisms at widely separated points geographically, indicates the possibility of a relationship. There is the possibility of a secondary relationship between the two organisms. It remains to be determined, however, if the diplobacillus is a specific or nonspecific secondary invader after the mycotic organism or vice versa.

It has not been demonstrated that the mycotic organism can be transmitted from one fish to another since in every observation here reported the granuloma may have been introduced by feeding infectious material. It is also possible that all the diseased fish were progeny from the same parent stock at the Berlin, New Hampshire Station, and were shipped as eggs to the New York and West Virginia stations. Likewise, it is not possible to attribute any mortality to the granuloma since large scale losses have always been associated with the diplobacillus. It may be speculated, however, that an insidious disease of this nature may well be correlated with the noted rise of fish mortality in hatcheries of the eastern seaboard over the last ten years.

It is interesting to note a profound change in fish cultural technique which may be a clue to the present observations. Since about 1925, fish producers have added an increasingly greater percentage of dry feed products to their production diets. These ingredients include a variety of the brewer's yeasts, torula yeast (*Torulopsis utilis*, a byproduct of the sulphite pulp industry), distiller's solubles, wheat middlings, cottonseed meal, and many types of fish meal. The fish meals are composed of several species of marine fish. To safeguard the nutritional value of these meals, low temperature drying is preferred. It is apparent that a potential source of yeastlike organisms exists. In view of the rather marked distribution of lesions in the anterior portion of the digestive tract, this aspect of the problem deserves further investigation.

## SUMMARY

1. A mycosis-like granuloma of fish has been described. The organism somewhat resembles the blastomycetes in tissue sections.

2. A gram-positive diplobacillus is consistently found in necrotic kidney lesions in conjunction with the granuloma.

3. The possible relationship between the mycotic granuloma and diplobacillus is discussed.

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